Université de Montréal

# Études sur la dérégulation des cytokines et des cellules Natural Killer chez les patients infectés par le VIH-1

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# Études sur la dérégulation des cytokines et des cellules Natural Killer chez les patients infectés par le VIH-1

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***That which does not kill us makes us stronger***

***Friedrich Nietzsche***

***La chance aide parfois, l’intelligence souvent, le travail toujours***

***Proverbe Brahman***

# Table des matières

Résumé ....................................................................................................... i
Abstract ...................................................................................................... iii
Abréviations ................................................................................................ v
1- Virus de l'Immunodéficience Humaine (VIH) ............................................... 1
1-1 SIDA et VIH ............................................................................................ 1
1-1-1 Découverte des virus de l'immunodéficience ............................................ 1
1-1-2 Pathogénèse associée aux VIH ................................................................ 2
1-2 Diversité des virus d'immunodéficience ....................................................... 3
1-3 Origine des VIH ........................................................................................ 4
1-4 Le VIH : Structure et génome ..................................................................... 6
1-4-1 Organisation génique .............................................................................. 6
1-4-2 Structure de la particule virale ................................................................ 7
1-5 Cycle réplicatif du VIH ............................................................................. 10
1-5-1 L'entrée virale ..................................................................................... 10
1-5-2 La transcription inverse et l'intégration .................................................. 10
1-5-3 L'expression du génome ........................................................................ 13
1-5-4 L'assemblage et le bourgeonnement ........................................................ 15
1-6 La thérapie antirétrovirale ......................................................................... 16
1-7 Développement d'un vaccin ........................................................................ 18
2- L'interleukine-21 (IL-21) ............................................................................ 19
2-1 Structure et expression de l'IL-21 ............................................................... 19
2-2 Le récepteur à l'IL-21 ................................................................................ 23
2-3 Effets biologiques de l'IL-21 sur les cellules immunitaires ............................... 26
2-3-1 Effet de l'IL-21 sur les cellules B ............................................................ 26
2-3-2 Effet de l'IL-21 sur les cellules T CD4$^{+}$ ............................................. 32
2-3-3 Effet de l'IL-21 sur les cellules Th17 ....................................................... 35
2-3-4 Effet de l'IL-21 sur l'induction des cellules T régulatrices ............................ 39
2-3-5 Effet de l'IL-21 sur les lymphocytes T CD8$^{+}$ ........................................ 39

2-3-6 Effet de l'IL-21 dans la génération des cellules Th folliculaires ............... 41
2-3-7 Effet de l'IL-21 sur les cellules Natural Killer ............... 42
2-3-8 Effet de l'IL-21 sur les cellules dendritiques ............... 44
2-3-9 Effet de l'IL-21 sur les cellules NKT ............... 44
2-3-10 Effet de l'IL-21 sur les macrophages ............... 47
2-4 Rôle de l'IL-21 dans l'auto-immunité et les maladies inflammatoires ............... 48
2-4-1 Rôle de l'IL-21 dans les maladies auto-immunes ............... 48
2-4-2 Rôle de l'IL-21 dans les maladies inflammatoires ............... 51
2-5 Rôle de l'IL-21 dans l'allergie et l'asthme ............... 53
2-6 Rôle de l'IL-21 dans la réponse anti-tumorale ............... 55
2-7 Rôle de l'IL-21 dans le contrôle des infections virales chroniques ............... 60
3- L'interleukine-18 (IL-18) ............... 65
4- Les cellules Natural Killer, leur biologie et leurs déterminants génétiques dans la résistance au VIH-1 ............... 68
5- Les cellules Natural Killer et l'infection par le VIH-1: conséquences sur l'élaboration de stratégies thérapeutiques et la vaccination. ............... 70
6- Mise à jour de la littérature concernant les cellules NK et l'infection par le VIH-1 ... 72
6-1 Interaction entre les cellules NK et les autres immunocytes ............... 72
6-2 Régulation des récepteurs et des ligands ............... 74
6-3 Perturbation de l'homéostasie des sous-types de cellules NK ............... 76
6-4 Les cellules Natural Killer utérines ............... 77
6-5 Études épistatiques sur les KIR et le CMH-I ............... 77
7- Dérégulation dans la production de cytokines lors de l'infection par le VIH-1 et le développement du SIDA ............... 79
7-1 L'interleukine-2 dans l'infection par le VIH-1 ............... 80
7-2 L'interleukine-4 dans l'infection par le VIH-1 ............... 81
7-3 L'interleukine-7 dans l'infection par le VIH-1 ............... 82
7-4 L'interleukine-10 dans l'infection par le VIH-1 ............... 86
7-5 L'interleukine-12 dans l'infection par le VIH-1 ............... 88
7-6 L'interleukine-15 dans l'infection par le VIH-1 ............... 89
7-7 Le TGF-β dans l'infection par le VIH-1 ............... 91
7-8 Le traitement anti-rétroviral (HAART) et la thérapie par les cytokines ............... 92

8- Contexte de l’étude ........ 96
9- Hypothèses émisent lors de l’étude ........ 97
9-1 Hypothèses concernant l’interleukine-21 ........ 97
9-2 Hypothèses concernant l’interleukine-18 ........ 97
10- Objectifs de l’étude ........ 98
11- Résultats concernant les études sur l’IL-18 ........ 99
11-1 Effet de l’IL-18 sur l’homéostasie des cellules NK lors de l’infection ........ 99
11-2 Déséquilibre entre l’IL-18 et l’IL-18BP lors de l’infection ........ 101
12- Résultats concernant les études sur l’IL-21 ........ 103
12-1 Régulation de l’IL-21 lors de l’infection par le VIH-1 ........ 103
12-2 Effet de l’IL-21 sur les cellules Natural Killer et la réplication du VIH-1 ........ 106
13- Discussion ........ 108
13-1 Discussion sur les études concernant l’IL-18 ........ 108
13-2 Discussion sur les études concernant l’IL-21 ........ 113
14- Conclusions ........ 126
15- Perspectives ........ 129
16- Références bibliographiques ........ 130
17- Articles publiés mais non inclus dans la thèse de doctorat ........ 175

## Liste des Figures


Figure 1. Évolution sommaire du système immunitaire de la primo-infection au stade du SIDA ........................................ 5

Figure 2. Organisation génomique des lentivirus de primates ........................................ 8

Figure 3. Structure et composition du VIH ........................................ 9

Figure 4. Cycle de réplication du VIH dans les cellules T $CD4^+$ ........................................ 12

Figure 5. Récepteurs des cytokines constitués d'une chaîne commune $\gamma$ ($\gamma_c$) ........................................ 22

Figure 6. Signalisation médiée par l'IL-21 après liaison à son récepteur ........................................ 24

Figure 7. Effet de l'IL-21 sur les cellules B ........................................ 34

Figure 8. Effet de l'IL-21 sur les cellules T ........................................ 36

Figure 9. Effet de l'IL-21 sur le développement des cellules Th17 ........................................ 37

Figure 10. Effets de l'IL-21 sur la réponse immunitaire médiée par les cellules T et les cellules Natural Killer ........................................ 45

Figure 11. Schéma résumant les différents effets de l'IL-21 sur les cellules immunes... 46

Figure 12. Conséquences et implications des effets biologiques de l'IL-21 sur les différentes composantes du système immunitaire ........................................ 61

Figure 13. Schéma récapitulant l'ensemble des découvertes réalisées sur la dérégulation de l'IL-18 et de l'IL-21 lors de l'infection par le VIH-1 et leurs conséquences sur le système immunitaire de l'hôte infecté. ........................................ 122

# Résumé

La prolifération, la différenciation ainsi que les fonctions des cellules du système immunitaire sont contrôlées en partie par les cytokines. Lors de l'infection par le VIH-1, les défauts observés dans les fonctions, la maintenance, ainsi que la consistance des cellules du système immunitaire sont en large partie attribués à une production altérée des cytokines et à un manque d'efficacité au niveau de leurs effets biologiques. Durant ces études, nous nous sommes intéréssés à la régulation et aux fonctions de deux cytokines qui sont l'IL-18 et l'IL-21.

Nous avons observé une corrélation inversée significative entre les concentrations sériques d'IL-18 et le nombre des cellules NK chez les patients infectés par le VIH-1. Nos expériences *in vitro* ont démontré que cette cytokine induit l'apoptose des cellules NK primaires et que cette mort peut être inhibée par des anticorps neutralisants spécifiques pour FasL et TNF-α. Cette mort cellulaire est due à l'expression de FasL sur les cellules NK et à la production de TNF-α par ces cellules. L'IL-18 augmente aussi la susceptibilité à la mort des cellules NK par un stimulus pro-apoptotique, en diminuant l'expression de la protéine anti-apoptotique Bcl-$X_L$.

Nous démontrons aussi que, contrairement à l'IL-18, les niveaux d'IL-18BP sont plus faibles dans les sérum de patients infectés. Ceci résulte sur une production non coordonnée de ces deux facteurs, aboutissant à des niveaux élevés d'IL-18 libre et biologiquement active chez les patients infectés. L'infection de macrophages *in vitro* induit la production d'IL-18 et réduit celle d'IL-18BP. De plus, l'IL-10 et le TGF-β, dont les concentrations sont élevées chez les patients infectés, réduisent la production d'IL-18BP par les macrophages *in vitro*. Finalement, nous démontrons que l'IL-18 augmente la réplication du VIH-1 dans les lymphocytes T $CD4^+$ infectés. Les niveaux élevés d'IL-18 libres et biologiquement actives chez les patients infectés contribuent donc à l'immuno-pathogénèse induite par le VIH-1 en perturbant l'homéostasie des cellules NK ainsi qu'en augmentant la réplication du virus chez les patients.

Ces études suggèrent donc la neutralisation des effets néfastes de l'IL-18 en utilisant son inhibiteur naturel soit de l'IL-18BP exogène. Ceci permettrait de moduler l'activité de l'IL-18 *in vivo* à des niveaux souhaitables.

L'IL-21 joue un rôle clef dans le contrôle des infections virales chroniques. Lors de ces études, nous avons déterminé la dynamique de la production d'IL-21 lors de l'infection par le VIH-1 et sa conséquence sur la survie des cellules T $CD4^+$ et la fréquence des cellules T $CD8^+$ spécifiques au VIH-1. Nous avons démontré que sa production est compromise tôt au cours de l'infection et que les concentrations d'IL-21 corrèlent avec le compte de cellules T $CD4^+$ chez les personnes infectées. Nos études ont démontré que le traitement antirétroviral restaure partiellement la production d'IL-21. De plus, l'infection par le VIH-1 de cellules T $CD4^+$ humaines inhibe sa production en réduisant l'expression du facteur de transcription c-Maf. Nous avons aussi démontré que la fréquence des cellules T $CD4^+$ spécifiques au VIH-1 qui produisent de l'IL-21 est réduite chez les patients virémiques. Selon nos résultats, l'IL-21 empêche l'apoptose spontanée des cellules T $CD4^+$ de patients infectés et l'absence d'IL-21 réduit la fréquence des cellules T $CD8^+$ spécifiques au VIH-1 chez ces patients.

Nos résultats démontrent que l'IL-21R est exprimé de façon égale sur tous les sous-types de cellules NK chez les donneurs sains et chez les patients infectés. L'IL-21 active les protéines STAT-3, MAPK et Akt afin d'augmenter les fonctions effectrices des cellules NK. L'activation de STAT-3 joue un rôle clef dans ces fonctions avec ou sans un traitement avec de l'IL-21. L'IL-21 augmente l'expression des protéines anti-apoptotiques Bcl-2 et Bcl-$X_L$, augmente la viabilité des cellules NK, mais ne possède aucun effet sur leur prolifération. Nous démontrons de plus que l'IL-21 augmente l'ADCC, les fonctions sécrétrices et cytotoxiques ainsi que la viabilité des cellules NK provenant de patients chroniquement infectés par le VIH-1. De plus, cette cytokine semble présenter ces effets sans augmenter en contrepartie la réplication du VIH-1. Elle permet donc d'inhiber la réplication virale lors de co-cultures autologues de cellules NK avec des cellules T $CD4^+$ infectées d'une manière dépendante à l'expression de perforine et à l'utilisation de la protéine LFA-1. Les niveaux d'IL-21 pourraient donc servir de marqueurs biologiques pour accompagner les informations sur le taux de cellules T $CD4^+$ circulantes en nous donnant des informations sur l'état de fonctionnalité de ce compartiment cellulaire. De plus, ces résultats suggèrent l'utilisation de cette cytokine en tant qu'agent immunothérapeutique pour restaurer les niveaux normaux d'IL-21 et augmenter la réponse antivirale chez les patients infectés par le VIH-1.

# Abstract

The proliferation, differentiation, function and maintenance of immune cells is controlled in large part by cytokines. HIV-induced dysfunctions of the antiviral immunity is in part related to defects in the cytokine network, as manifested by altered cytokine secretion and responsiveness to these cytokines. In these studies, we investigated the regulation and the functions of two cytokines, IL-18 and IL-21, during HIV-1 infection.

In our studies, we observed an inverse correlation between IL-18 concentrations and absolute numbers of various subsets of NK cells in infected persons. IL-18 caused increased death of a human NK cell line, as well as of primary human NK cells *in vitro*. The IL-18-mediated cell death was dependent upon Fas-FasL interactions and TNF-α secretion. IL-18 induced the expression of TNF-α, induced the expression of FasL on NK cells, increased the transcription from the human FasL promoter, reduced the expression of Bcl-$X_L$ in NK cells, and increased their sensitivity to FasL-mediated cell death. In contrast to IL-18 levels, IL-18BP levels decreased in the serum of HIV-infected patients. This decrease resulted in enhanced levels of free IL-18 in the serum of such patients. The infection increased production of IL-18 but decreased that of IL-18BP in monocyte-derived macrophages (MDM). Furthermore, IL-10 and TGF-β, two cytokines for which concentrations are increased in HIV-infected persons, also decreased production of IL-18BP by human MDM. Finally, recombinant human IL-18 enhanced HIV-1 replication in human $CD4^+$ T cells. The uncoordinated production of these two cytokines represents an imbalance between these two soluble factors in HIV-infected patients. Our study shows that enhanced IL-18 bioactivity in HIV-infected patients may contribute to the pathogenesis of AIDS by disrupting NK cell homoeostasis and increasing viral replication. This uncoordinated production of IL-18 and IL-18BP contribute to IL-18-induced immunopathology and pathogenesis in HIV-infected AIDS patients. Therefore, these studies suggest that the neutralization of IL-18 may represent an appropriate and useful immunotherapeutic strategy in these patients. It may delay AIDS progression and improve the immune status of infected persons. The best way to achieve this goal may be using exogenous interleukin-18 binding protein.

IL-21 is a relatively newly discovered immune enhancing cytokine, which plays an essential role in controlling chronic viral infections. Therefore, we sought to determine the dynamics of the cytokine production and its potential consequences on the viability of $CD4^+$ T cells in HIV-infected persons. We show here that the cytokine production is compromised early in the course of the infection. The serum cytokine concentrations correlated with $CD4^+$ T cell counts in the infected persons. Among different groups of HIV-infected persons, only Elite Controllers maintain normal production of the cytokine. The HAART partially restores production of this cytokine. Interestingly, HIV-1 infection of human PBMC as well as of purified $CD4^+$ T cells inhibits the production of the cytokine by decreasing the expression of c-Maf, a transcription factor involved in the activation of the cytokine gene, in the virus-infected cells but not in uninfected bystander cells. We also show that the frequencies of IL-21 producing HIV-specific antigen experienced $CD4^+$ T cells are decreased in HIV-infected viremic patients. Furthermore, we show that recombinant human IL-21 acts as pro-survival factor and prevents enhanced spontaneous apoptosis of *ex vivo* cultured $CD4^+$ T cells from HIV-infected patients and that increased serum levels of the cytokine are associated with higher frequencies as well as with better functions of HIV-specific CTL in HIV-infected individuals. We show that the cytokine receptors are expressed equally on all NK cell subsets. We demonstrate that the cytokine activates STAT-3, MAPK and Akt to enhance NK cell functions. IL-21 increases expression of anti-apoptotic proteins Bcl-2 and Bcl-$X_L$, and enhances viability of NK cells, but has no effect on their proliferation. We further show that the cytokine enhances HIV-specific ADCC, secretory and cytotoxic functions as well as viability of NK cells from HIV-infected persons. Furthermore, it exerts its biological effects on NK cells with minimal enhancement of HIV-1 replication, and the cytokine-activated NK cells inhibit viral replication in co-cultured HIV-infected autologous $CD4^+$ T cells in a perforin- and LFA-1-dependent manner. These studies suggest that serum IL-21 concentrations may serve as useful biomarker to accompany $CD4^+$ T cell counts for monitoring HIV-1 disease progression and the fitness of the antiviral immunity. Furthermore, the cytokine may be considered for immunotherapy in HIV-infected patients in order to restore the physiological levels of the cytokine and promote their antiviral immunity.

# Abréviations

| | |
|---|---|
| **ADCC** | *Antibody-Dependent Cellular Cytotoxicity* |
| **ADNc** | Acide Desoxy-Ribonucléique complémentaire |
| **AICD** | *Activation-Induced Cell Death* |
| **AID** | *Activation-Induced Cytidine Deaminase* |
| **ARN** | Acide Ribonucléique |
| **ARNm** | Acide Ribonucléique messagé |
| **ART** | *Antiretroviral Therapy* |
| **BCR** | *B Cell Receptor* |
| **CMH** | Complexe Majeur d'Histocompatibilité |
| **CD** | Cellule Dendritique |
| **CRFs** | *Circulating Recombinant Forms* |
| **CTS** | *Central Terminaison Sequence* |
| **LTC** | Lymphocyte T Cytotoxique |
| **Env** | *Envelope* |
| **Gag** | *Group Antigen* |
| **GM-CSF** | *Granulocyte-Macrophage Colony Stimulating Factor* |
| **HAART** | *Highly Active Anti-Retroviral Therapy* |
| **HCMV** | *Human Cytomegalovirus* |
| **HTLV** | *Human T-Cell Leukemia Virus* |
| **ICE** | *IL-1β-Converting Enzyme* |
| **ICOS** | *Inducible Costimulatory Molecule* |
| **IL** | Interleukine |
| **IL-18BP** | *Inteleukin-18 Binding Protein* |
| **INF** | Interféron |
| **KIR** | *Killer-cell Immunoglobuline-like Receptors* |
| **LAV** | *Lymphadenopathy-Associated Virus* |
| **LCMV** | *Lymphocytic Choriomeningitis Virus* |
| **LPS** | Lipopolysaccharide |
| **LTNP** | *Long Term Non Progressors* |

| | |
|---|---|
| **LTR** | *Long Terminal Repeat* |
| **MIP** | *Macrophage Inflammatory Protein* |
| **NCR** | *Natural Cytotoxicity Receptors* |
| **Nef** | *Negative Factor* |
| **NK** | *Natural Killer* |
| **NKT** | *Natural Killer T cells* |
| **PBMC** | *Peripheral Blood Mononuclear Cells* |
| **PHA** | Phytohemaglutinine |
| **Pol** | Polymerase |
| **PPT** | *Polypurine Tract* |
| **Rev** | *Regulator of viral gene expression* |
| **RRE** | *Rev Responsive Element* |
| **SOCS** | *Suppressor of Cytokine Signaling* |
| **SCF** | *Stem Cell Factor* |
| **SIDA** | Syndrome d'Immunodéficience Acquise |
| **Tat** | *Transcriptional activator* |
| **TAR** | *Trans-Activation Responsive Region* |
| **TCR** | *T Cell Receptor* |
| **TLR** | *Toll-like Receptor* |
| **Th1** | Lymphocyte T helper de type 1 |
| **Th2** | Lymphocyte T helper de type 2 |
| **Th17** | Lymphocyte T helper de type 17 |
| **TGF** | *Transforming Growth Factor* |
| **TNF** | *Tumor Necrosis Factor* |
| **TRAIL** | *TNF-Related Apoptosis-Induced Ligand* |
| **Trég** | Cellules T régulatrices |
| **VIH** | Virus de l'Immunodéficience Humaine |
| **VIS** | Virus de l'Immunodéficience Simienne |
| **Vif** | *Viral Infectivity Factor* |
| **Vpr** | *Viral protein R* |
| **Vpu** | *Viral protein U* |

# 1- Virus de l'Immunodéficience Humaine (VIH)

## 1-1 SIDA et VIH

### 1-1-1 Découverte des virus de l'immunodéficience

Les premiers cas de ce que l'on appelera plus tard le Syndrome d'Immuno-Déficience Acquise ou SIDA ont été décrits en 1981 chez 4 hommes homosexuels de Los Angeles [1]. Ces hommes présentaient un tableau clinique particulièrement sévère, associant une immunodéficience à de multiples infections virales et bactériennes. L'un de ces patients présentait un sarcome de Kaposi, maladie extrêmement rare et n'étant décrite à l'époque que chez des sujets âgés. Ce syndrome d'immunodéficience létal fut par la suite décrit dans d'autres villes américaines puis dans diverses régions du monde et, dès 1983, on a parlé d'épidémie [1, 2]. Cette même année, un rétrovirus fut isolé dans les ganglions lymphatiques de patients présentant les signes précurseurs du SIDA ou ayant développé un SIDA. Ce rétrovirus fut identifié comme étant l'agent causatif de ce syndrôme. Ce virus, apparenté aux Virus de Leucémie à Cellules T humaines (ou HTLV) fut dénommé LAV (pour *Lymphadenopathy-Associated Virus* [3]) ou HTLV-III [4], pour être finalement renommé Virus de l'Immunodéficience Humaine, ou VIH [5]. Quelques années plus tard, un nouveau rétrovirus fut isolé chez des patients présentant les signes cliniques du SIDA tout en ayant une sérologie VIH négative [6]. Ce virus, de structure et d'organisation génétique extrêmement proches de ceux du VIH, mais présentant une divergence de sérologie fut nommé VIH-2, tandis que le prototype initial pris la dénomination VIH-1. Par la suite, des virus apparentés aux VIH-1 et 2 ont été isolés chez des primates non-humains. Le premier rétrovirus simien fut isolé en 1985 dans une colonie de macaques au centre de primatologie de Nouvelle-Angleterre (États-Unis). Chez ces animaux, des symptômes analogues à ceux du SIDA humain firent soupçonner une infection rétrovirale. Ce tableau clinique fut rapidement associé à l'infection par un lentivirus nommé VISmac (pour Virus de l'Immunodéficience Simienne du macaque) [7]. Depuis lors, d'autres VIS ont été isolés chez différentes espèces de singes (voir classification au chapitre 1-3).

### 1-1-2 Pathogénèse associée aux VIH

Le SIDA mérite bien la dénomination de pandémie, désignant une épidémie qui se développe sur une large superficie en affectant une proportion importante de la population. Selon l'Organisation Mondiale de la Santé (OMS), le SIDA a, depuis son apparition, infecté plus de 47 millions d'individus, tué 22 millions de personnes et est devenu la quatrième cause de mortalité dans le monde. Plus de 95% des cas d'infection et de mortalité dus à l'infection par le VIH ont lieu dans des pays émergents. La grande majorité des souches responsables de la pandémie sont des isolats VIH-1, tandis que le VIH-2 n'est prévalent qu'en Afrique australe. L'évolution de l'infection par VIH-1 est typique des lentivirus [8] et peut être divisée en trois étapes (Figure 1) :

- **La primo-infection** : Elle peut être asymptomatique ou présenter des symptômes pseudogrippaux. Elle dure quelques semaines et se caractérise par une réplication intense du virus dans les organes lymphoïdes secondaires ainsi qu'une diminution du nombre de cellules T $CD4^+$ circulantes.

- **La phase de latence clinique ou asymptomatique** : Cette phase correspond à une activation de l'immunité humorale et cellulaire, particulièrement celle médiée par les cellules T $CD8^+$ cytotoxiques suite à la primo-infection. Cette réponse immunitaire permet de contrôler partiellement la réplication virale, aboutissant à une baisse sensible de la virémie. Un équilibre s'instaure alors entre le taux de réplication virale et la mortalité des lymphocytes T $CD4^+$, équilibre maintenu pendant plusieurs années, la durée variant en fonction de l'individu et de la souche virale. Le taux de réplication virale pendant cette phase est estimée à $10^9$ à $10^{10}$ virons produits par jour [9-11], tandis que le taux quotidien de renouvellement des lymphocytes T $CD4^+$ est de de l'ordre de $2.10^9$ [12, 13].

- **Le stade SIDA** : Cette phase correspond à une chute du nombre de lymphocytes T $CD4^+$ circulants, parallèlement à une hausse importante de la virémie. C'est pendant cette phase d'immunodéficience sévère qu'apparaissent des maladies opportunistes,

comme des pneumocystoses, des toxoplasmoses, pouvant être associées à des désordres neurologiques et/ou à des tumeurs comme le sarcome de Kaposi.

### 1-2 Diversité des virus d'immunodéficience

Les virus de l'immunodéficience humaine et simienne appartiennent à la famille des *retroviridae* et du genre lentivirus. Les rétrovirus se caractérisent par un génome composé de 2 copies d'ARN de polarité positive, rétro-transcrit suite à l'entrée dans la cellule cible grâce à une ADN polymérase virale, la transcriptase inverse. Au sein de cette famille, le genre lentivirus se distingue des autres rétrovirus par une organisation génomique complexe et par leur capacité à provoquer une infection persistante chez leurs hôtes.

Outre le VIH-1, le VIH-2 et le VIS, les lentivirus comptent les virus de l'immunodéficience féline (VIF) et bovine (VIB), ainsi que le virus Visna-Maedi chez le mouton, le virus de l'arthrite encéphalitique caprine (VAEC) et le virus de l'anémie infectieuse équine (VAIE).

Les VIS sont classés selon la nature de leur hôte naturel, chaque VIS étant dénommé selon l'espèce de singe chez laquelle il a été isolé. On distingue ainsi les virus infectant les macaques (VISmac), les singes verts africains (VISagm), les *sootey mangabeys* (VISsm), les mandrilles (VISmnd), les sytes (VISsyt) et les chimpanzés (VIScpz) [14].

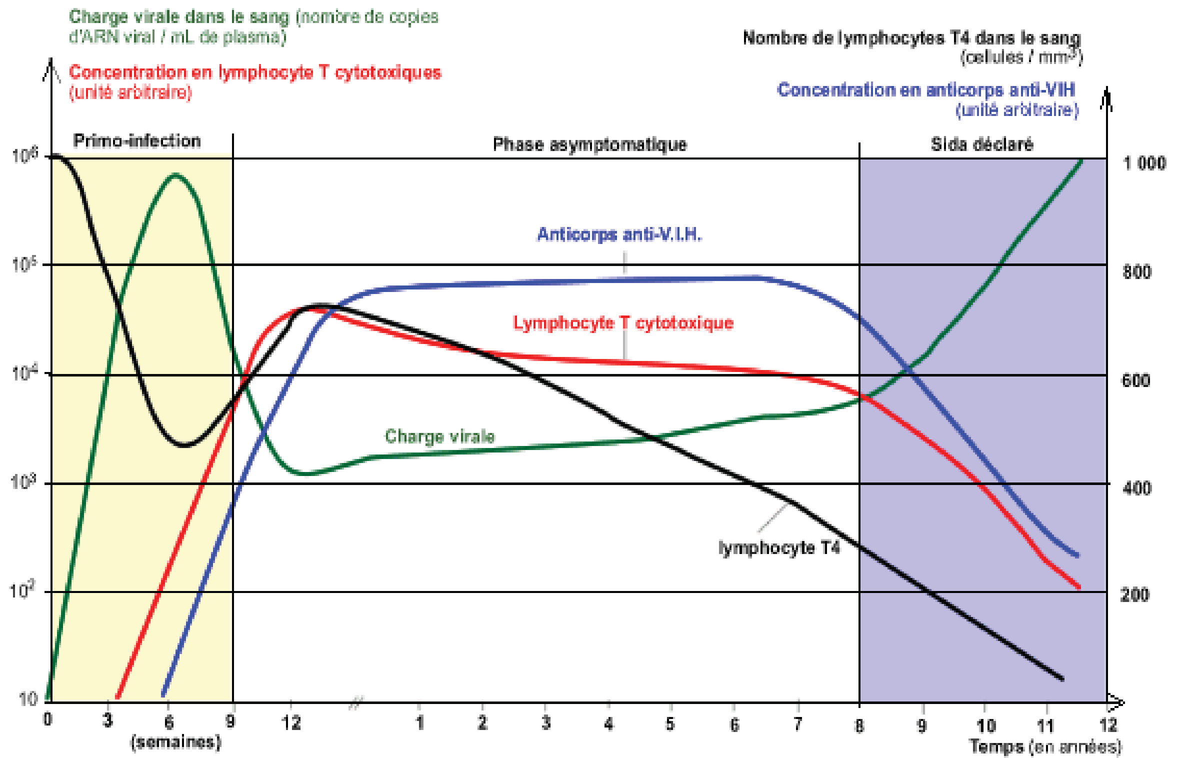
Les isoltas VIH-1 et VIH-2 sont eux aussi classés sur la base de leur organisation génomique et de leur proximité phylogénétique, respectivement en groupes et sous-types pour le VIH-1 et en sous-types pour le VIH-2. Ainsi, on compte 6 sous-types de VIH-2, notés de A à F, tandis que les souches de VIH-1 sont classées en trois grand groupes distincts : M, N et O.

La grande majorité des souches responsables de la pandémie appartient au groupe M (pour "*Major*" ou "*Main*") dans lequel l'analyse phylogénétique a permis d'identifier plusieurs sous-types. On dénombre actuellement 11 sous-types désignés de A à D, de F à H, J et K [15]. Les sous-types initialement désignés E et I ont été renommés respectivement CRF01-AE et CRF04-Cpx (CRF pour "*Circulating Recombinant Form*",

ou Forme Circulante Recombinante), leur génome étant issu de recombinaisons génétiques entre virus de différents sous-types.
Les isolats du groupe O (pour "*Outlier*") ont été décrits pour la première fois en 1990 [16] et sont très divergents du groupe M [17-19], notamment dans le gène d'enveloppe. Les virus du groupe O sont endémiques au Cameroun et dans les pays avoisinants mais, même dans cette région, ils restent très minoritaires.
Le groupe N (pour "*New*" ou "*Non-M, Non-O*") n'est représenté jusqu'à présent que par deux isolats de patients camerounais [20].
Cette classification reflète l'une des caractéristiques majeures des rétrovirus, à savoir leur très grande variabilité génétique. Cette variabilité est surtout due à la nature même de leur mode de réplication, fondé sur la transcription inverse. En effet, la transcriptase inverse est dépourvue d'activité exonucléase 3'-5' et réalise un taux d'erreur estimé à $10^{-4}$ à $10^{-5}$ par nucléotide incorporé [21]. A cette grande infidélité de la transcriptase inverse s'ajoutent le taux élevé de réplication virale chez les individus infectés [12] ainsi que la fréquence des événements de recombinaisons génétiques entre les génomes d'isolats distincts au sein d'un même individu [22].

### 1-3 Origine des VIH

Depuis leur découverte en 1981, la question de l'origine des VIH a fait l'objet de nombreuses contreverses. L'hypothèse d'une origine simienne fut rapidement favorisée dû à l'identification dès 1985 d'un rétrovirus phylogénétiquement proche du VIH-1 (le seul connu à cette époque), responsable d'un SIDA chez les singes macaques en captivité [7, 23, 24]. Ce virus portera plus tard la dénomination de VISmac.
Les données phylogénétiques, considérablement accrues depuis lors, ont permis de démontrer que les virus d'immunodéficiences sont entrés dans la population humaine lors de multiples infections inter-espèces (ou zoonoses) par des primates non-humains infectés par un VIS. Les souches de VIH transmises par des chimpanzés infectés par le VIScpz corespondent au VIH-1 tandis que celles transmises par les *sooty mangabeys* (VISsm) ont donné naissance au VIH-2 [14].




*(http://www.inrp.fr/Acces/biotic/immuno/images/courbeb.gif)*

**Figure 1. Évolution sommaire du système immunitaire de la primo-infection au stade du SIDA**

Ces données ont également permis d'estimer la date de ces transmissions inter-espèces. La souche de VIH la plus ancienne identifiée à ce jour correspond à un virus isolé à partir d'un échantillon de plasma d'origine africaine datant de 1959 [25]. Le positionnement de cet isolat dans l'arbre phylogénétique des virus d'immunoféficience de primates, au côté des sous-types B et D du groupe M de VIH-1, a permis d'estimer le début de la diversification entre les différents isolats du VIH-1 du groupe M entre 1915 et 1941. Cette estimation de la date d'apparition du premier ancêtre commun aux isolats VIH-1 du groupe M ne fournit pas de renseignements directs sur la date de transmission du virus entre chimpanzés et hommes. Selon les hypothèses, l'époque d'apparition de cette zoonose peut être estimée entre le début du XIXème siècle et 1930 [26, 27].

## 1-4 Le VIH : Structure et génome

### 1-4-1 Organisation génique

Le génome de l'isolat VIH-1 LAV initialement découvert en 1983 [3] a été cloné [28, 29] et séquencé, révélant un génome d'environ 9200 paires de bases (Figure 2). Comme tous les rétrovirus, le génome du VIH est constitué de 2 molécules d'ARN simple brin de polarité positive codant pour 3 gènes principaux :

- ***gag*** (pour "*group antigen*"), qui code pour les protéines de matrice (MA), de capside (CA) et de nucléocapside (NC).
- ***pol*** (pour "*polymerase*"), qui code pour les 3 enzymes virales que sont l'intégrase (INT), la protéase (PRO) et la transcriptase inverse (RT).
- ***env*** (pour "*envelope*"), qui code pour les glycoprotéines d'enveloppe virales (Env), constituées des sous-unités de surface (gp120 ou SU) et transmembranaire (gp41 ou TM).

Outre ces trois gènes principaux, caractéristiques des rétrovirus, les virus du genre lentivirus codent pour d'autres gènes appelés auxiliaires, bien que leur expression soit

essentielle au processus réplicatif [30, 31]. Ces gènes supplémentaires présents chez les lentivirus sont au nombre de 4 : *tat*. *nef*, *vif* et *rev*. A ces 7 gènes s'ajoutent *vpu*, présent chez le VIH-1 et le VIScpz, *vpx*, codé par le VIH-2, le VISsm, le VISmac et le VISagm et enfin *vpr*, codé par tous les lentivirus de primates, à l'exception de VISagm (Figure 2). Ce génome est encadré par des régions non-codantes qui donneront naissance, lors de l'étape de transcription inverse, à des séquences de même orientation, nommées LTR (pour "*Long Terminal Repeat*", voir chapitre 1-5-2 et 1-5-3).

### 1-4-2 Structure de la particule virale

Les particules du VIH apparaissent en microscopie électronique comme des structures sphériques d'environ 110 nm de diamètre. Les 2 molécules d'ARN constituant le génome viral sont associées à la protéine de nucléocapside. Ce complexe ARN / NC, ainsi que les 3 enzymes virales INT, PRO et RT sont contenus dans une capside constituée de la protéine CA. Cet ensemble, nommé *core*, constitue une structure conique et dense aux électrons dans les particules matures et infectieuses (Figure 3). Ce *core* est cerclé de protéines de matrice, elles-mêmes recouvertes d'une membrane de nature lipidique, issue de la membrane plasmique de la cellule infectée. Les glycoprotéines d'enveloppe sont ancrées dans cette bicouche lipidique, via la sous-unité transmembranaire gp41, associée à la gp120 par des liaisons non-covalentes. La stabilité de cette interaction est plus ou moins forte selon les isolats et la rupture de cette liaison, provoquant la perte de la sous-unités gp120, aboutit à une perte d'infectiosité [32].
Outre les composants principaux décrits, il a été montré que les virions renfermaient également les protéines Nef [33], Vpr [34], Vif [35] ainsi que des protéines cellulaires issues du cytosquelette de la cellule infectée [36]. A la surface des particules virales, des protéines d'origine cellulaire ont également été identifiées, comme les molécules du complexe majeur d'histocompatibilité (CMH) [37], la cyclophiline A [38, 39] ainsi que d'autres protéines.

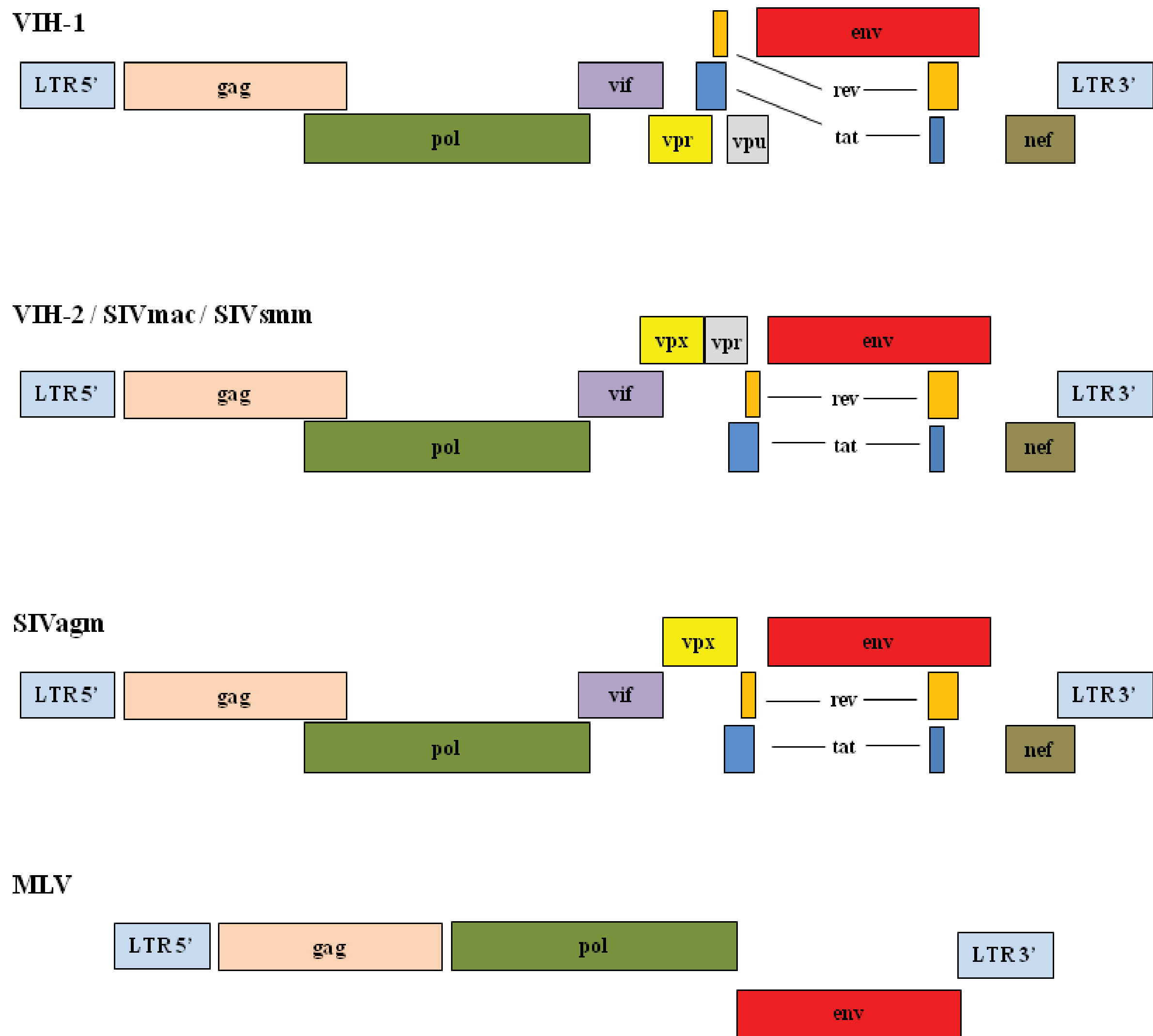
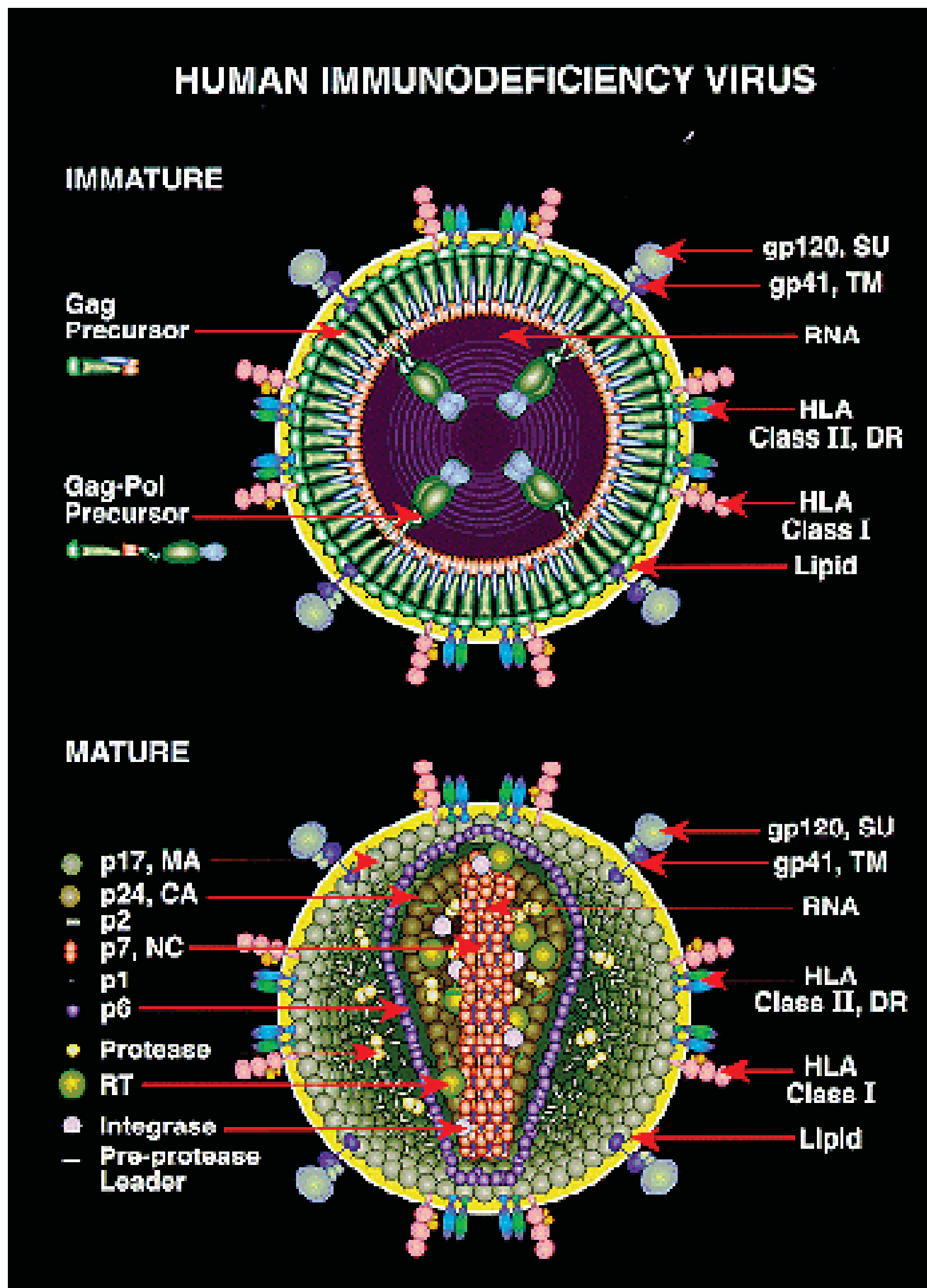



**Figure 2. Organisation génomique des lentivirus de primates**

L'organisation génomique des principaux représentants de virus d'immunodéficience de primates est représentée. Le génome d'un rétrovirus simple, MLV (*Murine Leukemia Virus*) est également indiqué à titre de comparaison. Les différents gènes sont représentés dans les 3 cadres de lecture et chaque génome est encadré par des LTR 5' et LTR 3'.




*(http://commons.wikimedia.org/wiki/File:Hiv.gif)*

**Figure 3. Structure et composition du VIH**

## 1-5 Cycle réplicatif du VIH

Les principales étapes du cycle réplicatif du VIH qui seront abordées dans ce chapitre sont représentées schématiquement sur la Figure 4.

### 1-5-1 L'entrée virale

Le processus d'attachement et d'entrée du VIH dans les cellules cibles est un processus complexe, qui a fait l'objet de très nombreuses études ces vingt-cinq dernières années. Le VIH est sans nul doute le virus dont le mécanisme d'entrée a été le plus éudié. Malgré cette recherche intensive, des interrogations persistent. Sommairement, c'est la glycoprotéine extérieure d'enveloppe, la gp120, qui est responsable de l'attachement des particules virales à la surface des cellules cibles, en interagissant avec des récepteurs (CD4) et co-récepteurs cellulaires (CCR5, CXCR4 et CCR2 principalement). Ces interactions conduisent à un remaniement conformationnel des glycoprotéines d'enveloppe, conduisant au démasquage d'une région hydrophobe de la gp41 dit "peptide de fusion". C'est l'ancrage de ce peptide dans la membrane plasmique de la cellule qui aboutit à la fusion des membranes virales et cellulaires, permettant la libération du core viral dans le cytoplasme. Ainsi, le processus d'entrée du VIH, contrairement à celui de la plupart des virus enveloppés, est indépendant du pH [40, 41].

### 1-5-2 La transcription inverse et l'intégration

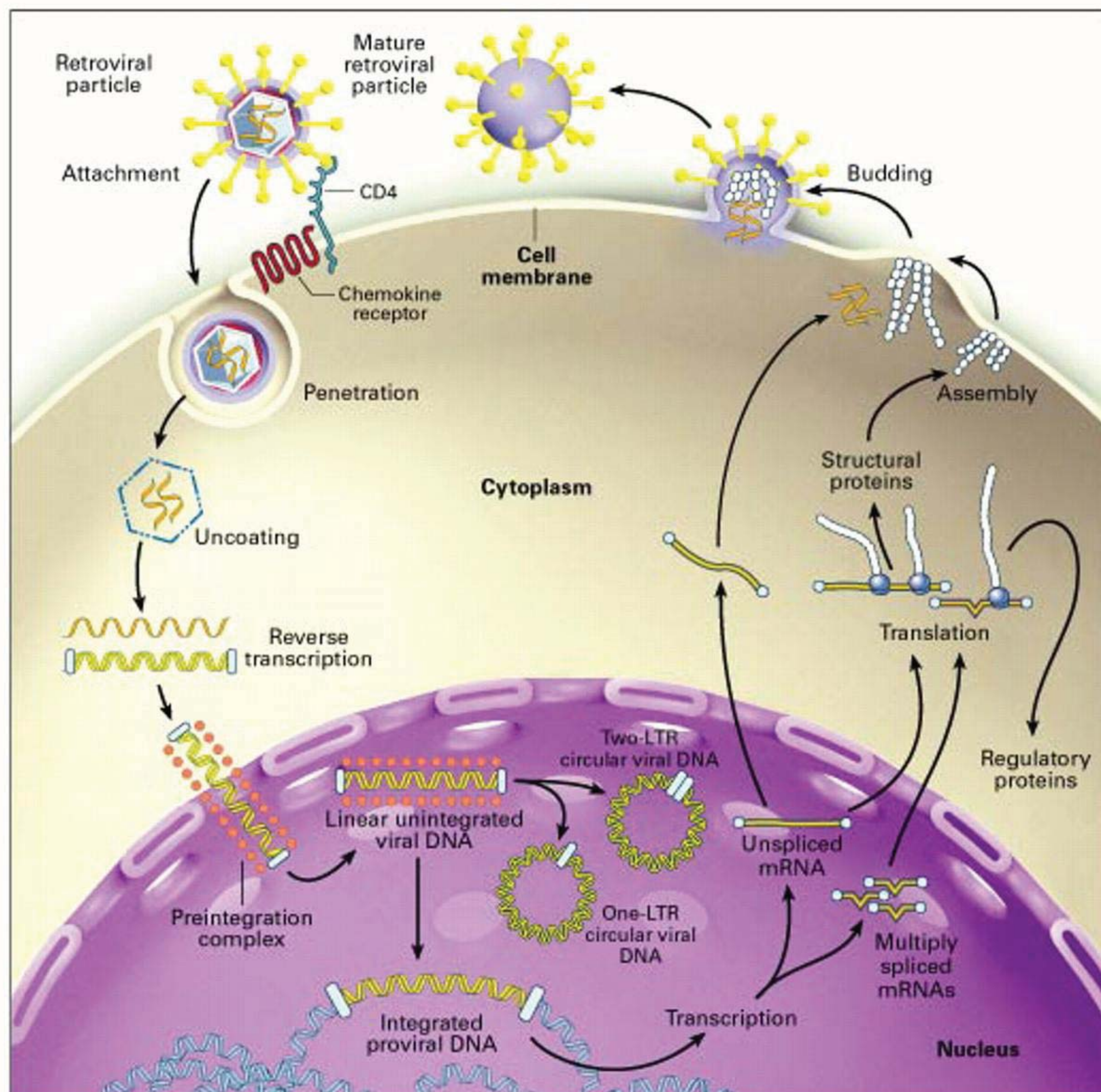
La transcription inverse est l'étape du cycle viral qui permet la synthèse d'un génome viral constitué d'ADN double-brin, nommé provirus, susceptible de s'intégrer au génome cellulaire, à partir des deux copies d'ARN simple-brin introduites dans le cytoplasme de la cellule infectée. Après l'étape d'entrée, le *core* viral subit une déstructuration partielle, selon un mécanisme mal caractérisé, conduisant notamment au relargage des protéines de capside CA et à une réorganisation des protéines de matrice MA autour du complexe ribonucléoprotéique [42]. Ce processus de "déshabillage" de

l'ARN viral permet notamment l'accés des désoxyribonucléotides triphosphates (dNTPs) cellulaires et la constitution du complexe de transcription inverse. Si la nature exacte de ce complexe nucléoprotéique est encore mal connue, le mécanisme de la réaction de transcription inverse, élucidé dans des systèmes acellulaires, est connu pour ne nécessiter que 4 constituants :

- La matrice, constituée des 2 copies d'ARN viral simple brin.
- L'amorce, constituée d'ARNt$^{lys}$ d'origine cellulaire et apportée par le virion.
- La RT virale, qui possède les deux activités essentielles à la réaction, transcriptase inverse et RNAse H.
- Des dNTPs, fournis par la cellule hôte.

Dans la cellule infectée, les microfilaments d'actine sont associés au complexe de transcription inverse, suggérant un rôle du cytosquelette cellulaire dans l'organisation de ce complexe [43].

La synthèse du premier brin d'ADN (brin négatif) par la transcriptase inverse est amorcée par l'ARNt$^{lys}$, associé à l'extrémité 5' de l'ARN viral, en bordure 3' de la région U5. Après avoir parcouru l'extrémité 5' de l'ARN, la transcriptase inverse poursuit la synthèse du brin ADN$^{-}$ à l'extrémité opposée de l'ARN viral. Ce premier "saut de brin" est à l'origine de la synthèse des LTR du provirus. Au cours de cette synthèse, l'activité RNAse H de la RT dégrade l'ARN de l'hybride ARN-ADN ainsi formé. La synthèse du deuxième brin d'ADN (positif) est initiée à partir d'amorces constituées de motifs riches en résidus purines nommés "polypurine tracts" (PPT). Ces motifs sont au nombre de 2, l'un en bordure de la région U3 du LTR 3' et l'autre, au centre du génome, nommé cPPT. La synthèse du deuxième brin d'ADN à partir de ces deux amorces nécessite un deuxième "saut de brin" et conduit à la formation de deux segments de taille identique, partiellement chevauchant. Ce court chevauchement de brins, nommé triplex central, est dû à un déplacement par la transcriptase inverse des 100 premiers nucléotides du brin d'ADN synthétisé à partir du cPPT [44]. Sa longueur est définie par la présence d'une séquence de terminaison de la transcription inverse dénommée CTS (pour "*Central Terminaison Sequence*").




*(Furtado et al. New Engl J Med.1999 )*

**Figure 4. Cycle de réplication du VIH dans les cellules T CD4+**

Ce triplex central, de structure très particulière, a été caractérisé comme un élément primordial de l'étape ultérieure de la transcription inverse soit l'import nucléaire [45].
Les lentivirus, contrairement aux autres rétrovirus, sont capables d'infecter des cellules quiescentes grâce à un processus d'import nucléaire indépendant de la mitose. Le passage à travers les pores nucléaires du complexe d'intégration, qui mesure approximativement la taille d'un ribosome, avec une masse de plusieurs millions de daltons, nécessite l'intervention de transporteurs [42]. Outre le triplex central, d'autres éléments semblent contribuer à ce mécanisme d'import et notamment la présence de sites d'adressage nucléaire dans les constituants du complexe de pré-intégration que sont les protéines Vpr , INT et MA [46-48].
L'intégration du génome ADN du VIH dans le génome cellulaire n'est pas spécifique d'un site précis. Elle met en jeu une molécule d'ADN double-brin linéaire, flanquée des deux LTR. Ses extrémités sont porteuses de séquences inversées répétées de 4 nucléotides reconnues spécifiquement par l'intégrase. Cette enzyme catalyse le clivage des deux derniers nucléotides 3' de chacun des deux brins ainsi que le clivage de l'ADN cellulaire en formant des extrémités cohésives 5' sortantes de 4 nucléotides. C'est une ligase cellulaire qui intervient pour joindre les 2 génomes. Le bilan de cette étape d'intégration est la suppression de 2 nucléotides à chaque extrémité de l'ADN viral et la duplication de 4 nucléotides de l'ADN cellulaire de part et d'autre du provirus intégré.

### 1-5-3 L'expression du génome

Une fois intégré dans le génome cellulaire, l'ADN proviral est transcrit. Les 9 gènes du VIH sont encadrés par les LTR, décomposés en 3 régions : U3, U5 (pour "*Untranslated regions*" 3' et 5') et R. Les LTR 5' et 3' étant orientés de façon identique (U3-R-U5), c'est leur position qui définit leur rôle [49].
- La transcription est initiée entre les régions U3 et R du LTR 5'. C'est la région U3 qui contient le promoteur de la transcription, composé de 3 domaines : le *core*, le domaine activateur (ou "*enhancer*") et le domaine modulateur. Le *core* contient les sites de fixation des facteurs de transcription comme la TATA-binding protein (TBP) ou SP-1.

Le domaine activateur est constitué de sites de fixation pour NF-κB. Enfin, le domaine modulateur contient des sites de fixation pour divers facteurs cellulaires comme AP-1, NFAT-1, USF-1, Ets-1, et LEF.

- La frontière entre les régions R et U5 du LTR 3' définit l'extrémité 3' de tous les transcrits viraux, la région R constituant le site de polyadénylation des ARN. L'expression du génome du VIH est complexe. Les 14 protéines virales sont traduites sur les 3 phases de lecture et sont exprimées grâce à des mécanismes d'épissage alternatif, de décalage du cadre de lecture et de maturation de précurseurs polyprotéiques. On peut décomposer la transcription du génome en 2 étapes, précoce et tardive:

- Durant la phase de transcription précoce, il y a tout d'abord synthèse d'ARN messagers doublement épissés codant pour Tat, Rev et Nef. C'est l'accumulation des protéines Tat et Rev qui va permettre la transcription des gènes de structure, lors de la phase tardive. En effet, Tat va trans-activer la transcritpion, en se fixant à un motif ARN en épingle à cheveux codé par la région R du LTR 5' et désignée TAR (pour "*Trans-Activation Responsive Region*"). La protéine Rev permet elle la stabilisation et l'export cytoplasmique des ARN messagés longs, mono- ou non-épissés, en reconnaissant une séquence située dans le gène *env* nommée RRE (pour "*Rev Responsive Element*").

- La phase tardive correspond donc à la formation des transcrits viraux longs, mono-épissés (vpu-env, vif et vpr) ou non-épissés (gag-pol). Les gènes *gag* et *pol* étant chevauchants, la traduction des transcrits non-épissés fait appel à un mécanisme original. Les transcrits sont traduits en un précurseur polyprotéique gag de 55 kDa, comprenant les protéines MA, CA et NC. Un événement de traduction sur 20 conduit à un décalage du cadre de lecture de -1, permettant la synthèse d'un précurseur gag-pol de 200 kDa. Ce mécanisme permet de moduler la stœchiométrie des protéines synthétisées, les protéines issues du gène *pol* et du gène *gag* étant ainsi produites dans une proportion de 1 pour 20. Le précurseur de 160 kDa des glycoprotéines d'enveloppe est quant à lui issu de l'épissage d'un transcrit commun à *env* et *vpu*.

### 1-5-4 L'assemblage et le bourgeonnement

L'assemblage des différents constituants des particules du VIH est une étape complexe du cycle de réplication, par laquelle les protéines, précurseurs de protéines et ARN viraux empruntent des voies d'acheminement différentes pour converger au niveau de la membrane plasmique où ils vont s'assembler de façon coordonnée [50].

Les produits du gène *gag*, c'est à dire les précurseurs polyprotéiques gag et gag-pol, traduits au niveau des polyribosomes libres, sont transportés directement à la membrane plasmique où a lieu leur auto-assemblage symétrique en une structure capsidique. Le mécanisme par lequel ces polyprotéines sont adressées sur le site d'assemblage est pour la plupart inconnu. On connaît en revanche l'implication du domaine MA dans ce processus d'adressage, de par la présence de sites de myristylation.

Durant l'assemblage des précurseurs gag et gag-pol, les molécules d'ARN génomiques, non-épissées, sont dimérisées et encapsidées par l'intermédiaire d'une séquence d'encapsidation et de dimérisation nommée "*psi*", située entre le LTR 5' et le cadre de lecture *gag*. C'est le domaine NC des précurseurs gag qui reconnaît cette séquence et permet l'encapsidation du génome viral.

Les précurseurs d'enveloppe gp160 sont quant à eux acheminés à la membrane plasmique par les voies classiques de sécrétion des protéines cellulaires. C'est durant ce cheminement que ces précurseurs seront clivés en deux sous-unités, gp41 et gp120 et que cette dernière sera glycosylée. Si la présence des glycoprotéines d'enveloppe n'est pas nécessaire à l'encapsidation, et au relargage des particules virales, leur présence à la surface des virus est indispensable pour leur conférer leur infectiosité. L'incorporation préférentielle des glycoprotéines d'enveloppe dans les virions en formation et l'exclusion de certaines protéines cellulaires sont encore partiellement inexpliquées. Elles serait notamment dues à des interactions entre le domaine MA des précurseurs gag et la sous-unités TM des glycoprotéines d'enveloppe. L'expression des glycoprotéines d'enveloppe virales à la surface des cellules infectés est responsable de leur capacité de fusion avec des cellules exprimant les récepteurs du VIH, aboutissant à la formation de cellules géantes, multinucléées nommées syncitia [51, 52].

Le bourgeonnement de la membrane plasmique s'effectue progressivement, parallèlement à l'assemblage progressif des constituants viraux. Pour effectuer cette extrusion, les précurseurs gag doivent interagir avec la bicouche lipidique. Les motifs miristylés du domaine MA seraient responsables de cette interaction, suite à un changement de conformation des précurseurs de capside. Une fois l'assemblage achevé, la dernière étape requise au relargage des particules virales consiste en un événement de fusion membranaire. Les particules ainsi libérées vont alors subir une maturation permettant de leur conférer leur infectiosité. Cette maturation consiste en la protéolyse des précurseurs gag et gag-pol, effectué par le domaine PRO, et en la réorganisation des protéines matures et des ARN viraux. C'est durant ce processus que se forme le *core* viral, dense aux éléctrons et que le virus devient une entité infectieuse.

### 1-6 La thérapie antirétrovirale

Plusieurs molécules antivirales ont été développées et approuvées depuis plusieurs années afin de stabiliser l'état de santé des personnes infectées. Il existe de nos jours six classes de médicaments utilisés dans la thérapie anti-rétrovirale qui ont pour unique but de stopper le virus à différentes phases de son cycle de réplication.
Les traitements anti-rétroviraux actuels consistent en la combinaison de plusieurs drogues ciblant diverses enzymes virales afin d'inhiber la réplication et ainsi réduire la virémie et le nombre de virus mutants chez les patients [53-56].
Bien que ces traitements permettent d'allonger l'espérance de vie des patients infectés, leur vie est profondément affectée par les multiples effets secondaires inhérents aux drogues utilisées comme la lipodystrophie ainsi que différent autres effets secondaires touchant différents organes. Les recherches actuelles cherchent à découvrir de nouveaux inhibiteurs possèdant une faible toxicité pour l'organisme. Les inhibiteurs existant permettent de bloquer la transcriptase inverse, la protéase, l'intégrase ainsi que d'empêcher le mécanisme de fusion permettant au virus d'entrer dans les cellules cibles.

La transcriptase inverse utilise les désoxyribonucléotides triphosphates (dNTPs) présent

dans la cellule hôte pour synthétiser l'ADN viral. Des analogues de nucléotides triphosphorylés mais dépourvus du groupement hydroxyl en 3' sont devenus de puissants anti-rétroviraux puisqu'ils provoquent la terminaison prématurée de la molécule d'ADN naissante. Cette classe de molécules est représentée par les NRTIs ou NtRTIs (*Nucleoside/Nucleotide Reverse Transcriptase Inhibitors*). Parmi ces analogues, on retrouve l'AZT et plus d'une quinzaine d'autres molécules actuellement utilisées ou en phase d'essai [57]. La deuxième classe d'anti-rétroviraux ciblant la RT sont les NNRTI (*Non Nucleoside Reverse Transcriptase Inhibitors*) comprenant des molécules utilisées en clinique et d'autres en phase d'essai. Les NNRTI sont des anti-rétroviraux allostériques qui inhibent la polymérisation de l'ADN viral en se fixant sur un site différent du site catalytique de l'enzyme [58, 59].

Les inhibiteurs de protéase sont des analogues peptidiques qui agissent en mimant une liaison phénylalanine-proline. En se fixant dans le site actif de la protéase, ils agissent comme inhibiteurs compétitifs du substrat naturel de l'enzyme [60, 61].

L'intégrase est une enzyme qui permet d'intégrer l'ADN proviral au génome de l'hôte. Cette enzyme a rapidement constitué une cible de choix dans la mesure où elle ne possède pas d'équivalent chez l'homme. Il existe trois inhibiteurs d'intégrase: le raltégravir et l'elvitégravir, tout deux commercialisés, ainsi que le MK-2048. Le MK-2048 est de nos jours en phase d'essai clinique de niveau II [62-65].

Le T20 (Enfuvirtide) est un inhibiteur de fusion peptidique. Le T20 se fixe sur la protéine gp41 et l'empêche de remplir sa fonction de fusion entre les membranes virales et cellulaires. Utilisé en injection sous-cutanée, le T20 assure une forte réduction de la charge virale [66, 67]. Le T20 a obtenu l'autorisation de mise sur le marché aux États-Unis en mars 2003 et est utilisé en combinaison avec d'autres anti-rétroviraux.

De nos jours, le traitement communément appelé "*Highly Active Antiretroviral Therapy*" (HAART) se donne en combinant trois médicaments contenant deux classes différentes d'agents anti-rétroviraux incluant au moins un inhibiteur de protéase. Ce traitement est

très efficace dans l'inhibition de la réplication virale [68]. Il restaure partiellement l'immunocompétence des patients et, par conséquent, ralentit la progression de l'infection vers le stade SIDA [68]. Il a des effets bénéfiques directs sur les différents paramètres du système immunitaire qu'ils soient inné ou adaptatif. Cette thérapie a permis au SIDA de devenir une infection chronique plus contrôlable. Cependant, il est très difficile de se conformer à la stringence de ce régime, ce qui résulte sur l'émergence de souches résistantes à ces médicaments [69].

### 1-7 Développement d'un vaccin

Aucun vaccin prophylactique efficace existe à ce jour pour cette infection. Les progrès dans le développement d'un vaccin sont très lents. Ceci étant dû à une grande capacité de mutation du virus aboutissant à une variation dans les souches virales sur la planète ainsi que la capacité du virus à échapper à la réponse immunitaire de l'hôte. Plusieurs concepts et stratégies vaccinales se poursuivent bien que les différents essais n'aient démontré jusqu'à présent aucune protection contre le virus chez les patients infectés [70].

## 2- L'interleukine-21 (IL-21)

La réponse immune à des corps étrangers est constituée de deux bras principaux qui sont l'immunité innée et l'immunité acquise ou adaptative. Ces deux réponses interagissent de façon coordonée dans l'organisme. Les composantes cellulaires de la réponse immune innée incluent les cellules dendritiques (CD), les cellules Natural Killer (NK), les macrophages et les granulocytes. Un des phénomènes clés du système immunitaire inné est la production de cytokines qui peuvent réguler la réponse immunitaire acquise médiée par les lymphocytes T et B. Ceci permettra le développement des réponses effectrices qui seront spécifiques à un antigène. Les cytokines produites par le système immunitaire inné, comme celles produites par les cellules T activées, vont donc permettre à la réponse immunitaire de se développer, d'acquérir ses fonctions effectrices, et vont permettre de désactiver ces réponses lorsque les corps étrangers seront éliminés. Beaucoup d'attentions ont été portées depuis plusieurs décénnies à ces cytokines, tant au niveau de la compréhension de leur fonctionnement et leur effet régulateur du système immunitaire, que sur leur potentiel clinique pour le traitement de patients atteints de cancers ou d'infections par des micro-organismes.

La famille des cytokines de type I, qui sont constituées de 4 hélices alpha en faisceaux, représente l'une des famille de cytokine les plus importantes. Cette famille contient une sous-famille de cytokines constituée d'interleukines, dont le récepteur est couplé à une chaîne commune gamma ($\gamma_c$) (Figure 5). Cette sous-famille comprend l'IL-2, l'IL-4, l'IL-7, l'IL-9, l'IL-15 et finalement l'IL-21, le membre de cette famille le plus récemment découvert [71, 72].

### 2-1 Structure et expression de l'IL-21

L'interleukine-21 (IL-21) est une cytokine relativement nouvelle qui a été découverte en 2000 par des scientifiques travaillant dans la compagnie Zymogenetics Inc. [73]. Le gène codant pour cette cytokine est situé sur le chromosome 4. L'ADNc de

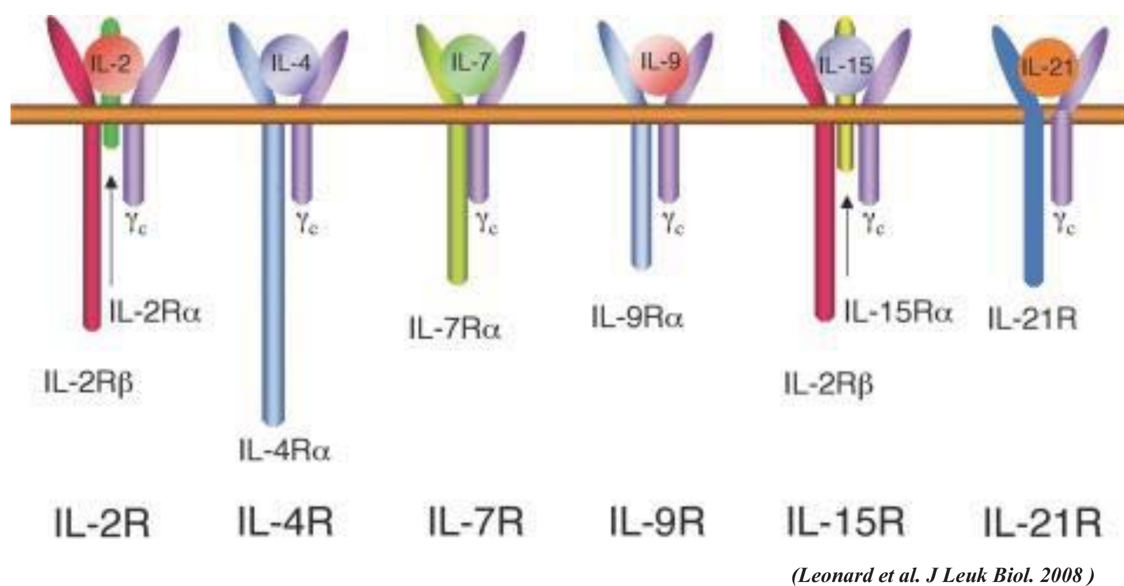
l'IL-21 code pour un polypeptide de 155 acides aminés permettant d'obtenir une protéine mature ayant une masse moléculaire de 18 kDa [73]. L'IL-21 est une cytokine de type I qui se lie à son récepteur (IL-21R) appartenant à la famille des récepteurs de cytokines de classe I. Ces récepteurs forment un complexe hétérodimérique avec les chaînes $\gamma$ communes, ce qui permet une signalisation intracellulaire après liaison avec leurs ligands appropriés. L'IL-21 est donc similaire aux cytokines de la même famille qui sont l'IL-2, l'IL-4, l'IL-7, l'IL-9 et l'IL-15, au niveau de l'interaction avec son récepteur [71] (voir Figure 5). Récemment, la structure tridimensionelle de l'IL-21 humaine a été résolue par spectroscopie à raisonnance magnétique nucléaire [74]. Sa structure correspond à une structure classique "*up-up-down-down*" de 4 hélices alpha en faisceaux. Un segment de la molécule impliquant une hélice C relativement instable est importante pour sa liaison au récepteur. Une stabilisation de cette région dans une IL-21 analogue a démontré une augmentation dix fois supérieure au niveau de son activité biologique [74].

Les travaux de Rahman et al. ont permis de découvrir un isoforme de l'IL-21 (IL-21iso) chez l'homme et la souris [75]. Cette IL-21iso serait obtenue par épissage alternatif, ce qui aboutirait à une IL-21 possèdant une région C-terminale différente de l'IL-21 normale. Cependant, les deux IL-21 semblent avoir les mêmes effets biologiques concernant la prolifération des cellules B et des cellules T ainsi que la production d'IFN-$\gamma$ par les cellules NK. Ces deux cytokines activeraient de façon similaire les protéines STAT-1 et STAT-3.

Une étude récente a aussi permi d'élaborer des analogues de l'IL-21 qui peuvent lier la chaîne $\alpha$ de l'IL-21R mais qui ont perdu leur affinité pour la chaîne commune $\gamma$. Les auteurs de cette étude ont prédit les résidus nécesaires permettant la liaison à la $\gamma_c$ et les ont mutés pour créer des IL-21 ne pouvant pas se lier à la $\gamma_c$. Leurs études fonctionelles sur ces mutants ont permis de pouver leur utilisation possible en tant que cytokines antagonistes à l'IL-21 sauvage [76].

L'IL-21 est principalement produite par tous les différents sous-types de lymphocytes T $CD4^+$ (voir le chapitre 2-3-2) ainsi que les cellules NKT [73, 77]. Bien que les gènes codant pour l'IL-21 et l'IL-2 soient adjacent, leur régulation est distincte. Ces deux cytokines peuvent être induites dans les cellules T $CD4^+$ en activant le récepteur des

cellules T (TCR). L'IL-21 peut être induite par un signal calcique seul contrairement à l'IL-2 dont l'expression requiert un signal calcique associé à l'activation d'une protéine Kinase C [78]. La région promotrice du gène de l'IL-21 contient un site de liaison pour le facteur nucléaire des cellules T activées (NFAT) qui permet sa régulation [78, 79]. Cependant, des souris qui n'expriment pas le facteur de transcrirption NFATC2 expriment tout de même l'IL-21, ce qui suggère l'utilisation d'autres facteurs de la famille NFAT ou bien d'autres facteurs de transcription pour réguler l'expression de cette cytokine [78]. Des études ont démontré que la perte de l'expression du facteur de transcription c-Maf résulte sur un défaut de la production d'IL-21 *in vivo* [80]. Ces études montrent que l'activation de la protéine ICOS (*Inducible Costimulatory Molecule*) ainsi que l'IL-6 augmentent l'expression de c-Maf dans les cellules T $CD4^+$, ce qui induit l'expression de l'IL-21 [80]. Les études menées par Hiramatsu et al. démontrent que c-Maf se lie directement au promoteur de l'IL-21 pour permettre son expression [81]. De plus, ils ont démontré que le *Transforming Growth Factor-β* (TGF-β) réduit l'expression de l'IL-21 induite par c-Maf dans les cellules T $CD4^+$ [81]. De façon similaire, une étude a démontré que l'IL-27, une cytokine reconnue pour générer des cellules T régulatrices de type 1 produisant de l'IL-10 (appelées cellules Tr1), induit l'expression de c-Maf, ICOS et la production d'IL-21 [82]. L'IL-27 permet donc de transactiver l'expression de l'IL-21 par l'intermédiaire de c-Maf. Cette IL-21 va ensuite, de façon autocrine, promouvoir le développement des cellules Tr1 [82]. Plusieurs études démontrent une régulation autocrine de l'IL-21 dans les cellules T humaines. L'IL-21 active STAT-3 qui se lie au promoteur du gène codant pour cette dernière. Ces études montrent aussi que l'IL-6, aussi reconnue pour activer STAT-3, active l'expression de l'IL-21 alors que le TGF-β inhibe sa production [83, 84]. Une étude a aussi démontré qu'un antigène mycobactérien (BCG) pouvait augmenter l'expression de l'IL-21 dans les cellules NKT de souris ou humaines, ce qui suggère une induction possible de l'IL-21 par des systèmes moléculaires sensibles aux corps étrangers présents sur les cellules du système immunitaire comme les *Toll-like Receptors* (TLR) [85]. Une étude récente a d'ailleurs démontré que l'activation du TLR3 par de l'acide polyinosinic:polycytidylic (poly(I:C)) augmente l'expression de l'IL-21 dans les cellules T $CD4^+$ naïves [86].

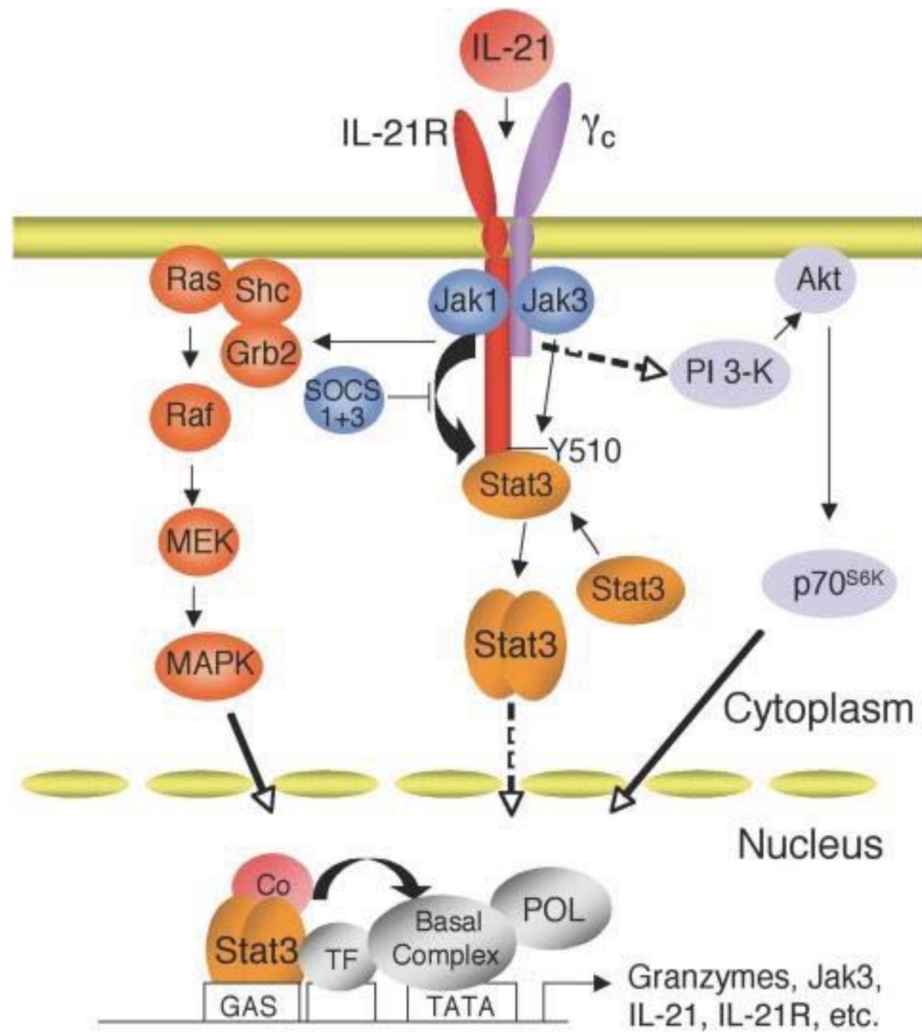



***(Leonard et al. J Leuk Biol. 2008 )***

**Figure 5. Récepteurs des cytokines constitués d'une chaîne commune γ ($\gamma_c$)**

**2-2 Le récepteur à l'IL-21**

Le récepteur à l'IL-21 (IL-21R) a été découvert en 2000 en tant que récepteur orphelin et a tout d'abord été appelé "NILR" pour "*Novel Interleukin Receptor*" [87, 88]. Il a été découvert par séquencage à partir d'ADN génomiques et complémentaires. Le gène codant pour ce récepteur se trouve immédiatement en amont du gène codant pour le récepteur à l'IL-4 sur le chromosome 16. Ce récepteur devient fonctionnel, comme c'est le cas de tous les récepteurs liant les cytokines de la même famille, lorsqu'il est associé à une chaîne $\gamma_c$ [89, 90]. L'IL-21 fut par la suite clonée et reconnue comme ligand pour ce récepteur [88]. Le domaine cytoplasmique de l'IL-21R contient 6 résidus tyrosines dont un en position 510 qui est phosphorylé et sert de port d'attache pour les protéines STAT-1 et STAT-3 (Figure 6) [91]. Des études ont aussi démontré que la voie des MAP kinases et celle de la PI3-kinase (PI3-K) sont impliquées dans la signalisation de l'IL-21 (Figure 6) [91]. La signalisation médiée par le récepteur à l'IL-21 implique la voie Jak-STAT comme c'est le cas de tous les membres de cette famille. Jak1 et Jak3 sont les tyrosines kinases de la famille Janus qui sont activées par l'IL-21 [90, 92]. Cette cytokine peut activer les protéines STAT-1, STAT-3, STAT-5a et STAT-5b [91, 93]. Cependant, l'activation de la protéine STAT-5 est faible et l'activation de STAT-3 est la plus importante dans la signalisation médiée par l'IL-21. En effet, il y a un défaut majeur dans la signalisation médiée par l'IL-21 dans les cellules T si il n'y a pas d'expression de STAT-3 [91]. L'étude menée par Kwon et al. a démontré que la majorité des régions qui lient le STAT-3 activé par l'IL-21 peuvent aussi se lier à l'IRF-4 (*Interferon Regulatory Factor-4*) [94]. Leur étude montre que les gènes régulés par l'IL-21 sont associés à des sites liant STAT-3 en combinaison avec l'IRF-4 plutôt que la protéine STAT-3 seule. Cette interaction semble d'ailleurs être importante pour l'expression du gène *Prdm1* qui code pour le facteur de transcription Blimp-1 (*B lymphocyte-induced maturation protein-1*), un facteur de transcription reconnu pour avoir un rôle clef dans l'homéostasie des cellules T et dans la différenciation des cellules plasmatiques. La liaison de STAT-3 activée par l'IL-21 sur les sites de régulation géniques est diminuée dans les cellules T provenant de souris KO pour l'IRF-4.




*(Leonard et al. J Leuk Biol. 2008 )*

**Figure 6. Signalisation médiée par l'IL-21 après liaison à son récepteur**

De même, la différenciation des cellules T folliculaires par l'IL-21 est inhibée *in vivo* dans des souris KO pour l'IRF-4 [94].

La protéine virale Tax1 du *Human T-cell Leukemia Virus type 1* (HTLV-1) transactive les gènes codant pour l'IL-21 et le récepteur à l'IL-21 (IL-21R) dans les cellules T [95]. Leur étude a permis de découvrir deux éléments de réponses à Tax1 dans les promoteurs de ces deux gènes. Leurs analyses ont révélé que les facteurs de transcriptions AP-1 (*Activating Protein-1*) et NF-κB (*Nuclear Factor-κB*) lient le promoteur de l'IL-21 et que AP-1 et les IRF (*Interferon Regulatory Factor*) lient celui de l'IL-21R [95].

Cette étude démontre donc l'importance de la coopération entre ces deux facteurs afin de moduler l'activation transcriptionelle lors d'une signalisation médiée par l'IL-21.

Une étude récente a permis de découvrir une molécule se liant à l'IL-21R nommée WSB-2 (WD-40 contenant des *SOCS-box-2*) [96]. Cette molécule se lie au niveau de la région intracytoplasmique du récepteur qui est située près de la membrane cellulaire. La sur-expression de cette molécule réduit l'expression de l'IL-21R et inhibe la signalisation médiée par l'IL-21. Il semble donc que cette molécule contrôle négativement l'expression de ce récépteur et inhibe la signalisation médiée par l'IL-21 [96].

L'IL-21R est exprimé principalement par les cellules T $CD4^+$, $CD8^+$, les cellules B, les cellules dendritiques, les macrophages, les cellules NK et les kératinocytes [87, 97-101]. L'IL-21 possède une action pléïotropique sur différents types de cellules ayant un rôle dans la réponse immunitaire. L'IL-21 ne possède pas de rôle clef dans l'hématopoïèse bien que l'expression de l'ARN messagé codant pour l'IL-21 a été détecté dans une population de progéniteurs de la moelle osseuse et que l'IL-21 peut permettre l'expansion de ces cellules progénitrices *in vivo* et *in vitro* [102, 103].

L'IL-21R est fortement exprimé au niveau des cellules B qu'elles soient au repos ou bien activées [87, 100]. Le récépteur à l'IL-21 est exprimé à faible niveau au stade cellule pré-B de leur développement et persiste à ce niveau au stade transitionel T1 pour ensuite augmenter au second stade transitionel T2 [104]. Les cellules B folliculaires matures expriment de plus haut niveaux d'IL-21R que les cellules T matures [100]. Ces niveaux sont de plus augmentés lors d'une activation par le récepteur aux cellules B (BCR) ou lors d'une activation médiée par le CD40 et son ligand CD40L [105]. Les

cellules B marginales répondent aussi à l'IL-21 bien qu'elles aient des plus faibles niveaux d'expressions d'IL-21R que les cellules folliculaires [104]. Les cellules plasmatiques n'expriment pas de façon détectable le récepteur à l'IL-21 [105].
Une plus faible expression de ce récepteur est observée sur les cellules T bien qu'elle puisse être augmentée lors d'une stimulation du récepteur des cellules T (TCR), qui induit la déphosphorylation du facteur de transcription Sp1 [100, 106]. Au niveau du thymus, le récepteur à l'IL-21 est induit à la surface des thymocytes entre le stade double négatif ($CD4^-CD8^-$) et le stade double positif ($CD4^+CD8^+$) [100], mais cette expression ne semble pas requise pour le développement thymique car un développement normal des lymphocytes T est observé chez les souris KO pour le récepteur à l'IL-21 [103].

### 2-3 Effets biologiques de l'IL-21 sur les cellules immunitaires

#### 2-3-1 Effet de l'IL-21 sur les cellules B

**L'IL-21 joue un rôle critique dans la production des immunoglobulines (Ig):**

Le rôle de l'IL-21 dans le fonctionnement des cellules B a été étudié tant au niveau *in vitro* que *in vivo* en utilisant des modèles de souris transgéniques pour l'IL-21 ou KO pour l'IL-21R (Figure 7). L'IL-21 n'est pas essentielle pour le développement des cellules B. Aucun défaut dans les différents sous-types de cellules B n'est observé chez les souris KO pour l'IL-21R tant au niveau de la moelle osseuse qu'en périphérie [103]. Les cellules B de souris KO pour l'IL-21R prolifèrent normalement en réponse au lipopolysaccharide (LPS), à un anti-CD40, ou à la combinaison d'IL-4 et d'anti-IgM [103]. Les souris KO pour l'IL-21R ont cependant un défaut dans la production d'IgG1 et une augmentation des niveaux sériques d'IgE [103]. Lors d'une immunisation avec des antigènes dépendant des cellules T, les souris KO pour l'IL-21R ont un défaut majeur dans la production d'IgG1 et une augmentation majeure d'IgE spécifiques aux antigènes [103]. Ces résultats sont surprenant car ces deux anticorps sont régulés de façon coordonnée. Les niveaux élevés d'IgE chez les souris KO pour l'IL-21R sont

consistants avec des expériences qui démontrent que l'IL-21 injectée à des souris sauvages conduit au moment de l'immunisation à une réponse IgE réduite, ainsi que des expériences qui montrent que l'IL-21 peut réduire le niveau d'expression du transcrit codant pour le Cε résultant sur un faible *switching* vers la production d'IgE [107]. Des études *in vitro* ont démontré que l'IL-21 peut réguler aussi bien positivement que négativement la production d'IgE à partir de cellules B de sang périphérique humain et ceci dépendamment du contexte. L'IL-21, en combinaison avec de la phytohaemaglutinine (PHA) et de l'IL-4, inhibe la production d'IgE alors que l'IL-21 associée avec des anticorps anti-CD40 et de l'IL-4 augmente la production de cette dernière [108]. L'étude menée par Avery et al. a démontré que cette augmentation de la production d'IgE dépendait du bon fonctionnement de STAT-3 [109]. Une autre étude menée par le même groupe a démontré que les personnes ayant des mutations qui inactivent la fonction de STAT-3 ont un nombre réduit de cellules B mémoires fonctionnelles et que leurs cellules B naïves ne peuvent plus se différencier en cellules plasmatiques en réponse à l'IL-21 [110]. Cependant, les personnes ayant des mutations dans la protéine STAT-1 possédaient des cellules B mémoires fonctionnelles et leurs cellules B répondaient à l'IL-21 [110]. Une autre étude démontre que l'ajout d'IL-10 et de cellules transfectées par le CD32 lors de cette activation des cellules B augmente l'expression de l'AID (*Activation-Induced Cytidine Deaminase*) [111]. Les mécanismes moléculaires précis régulant la production des Ig restent encore aujourd'hui mal connus, ceci étant particulièrement dû au fait que l'IL-21 peut avoir un rôle pro-apoptotique sur les cellules B.

**L'IL-21 induit l'apoptose des cellules B selon leur contexte d'activation:** Contrairement aux autres cytokines $\gamma_c$ dépendantes, l'IL-21 peut avoir un effet pro-apoptotique sur les cellules B (Figure 7). L'IL-21 augmente la prolifération des cellules B lorsqu'elle est associée à une stimulation par un anticorps anti-CD40 alors qu'elle l'inhibe en présence d'anti-IgM et d'IL-4 [73]. L'effet inhibiteur de l'IL-21 sur la prolifération des cellules B lors d'une stimulation par du LPS est en partie dû à son effet pro-apoptotique sur ces dernières [100, 112, 113]. Le degré d'apoptose est en relation avec le contexte d'activation des cellules B. Lorsque ces cellules sont activées avec des

signaux médiés par les *Toll-Like Receptors* (TLR) comme le LPS ou du CpG, l'IL-21 induit leur apoptose [100, 112, 113]. Cependant, l'IL-21 augmente leur prolifération lorsqu'elles sont activées par des signaux venant du récepteur des cellules B (BCR, comme celui émis par un anti-IgM), ainsi que par des signaux de co-stimulation dérivés des cellules T (anti-CD40) [100, 112, 113]. Le signal apoptotique est caspase-dépendant. Cependant, l'IL-21 n'a aucun effet sur les niveaux d'expression de la protéine Bcl-2 dans les cellules B [112, 113]. Par contre, l'IL-21 active l'ARN messager de la protéine pro-apoptotique Bim et réduit l'expression de Bcl-$X_L$ [100]. Des études montrent que Bim-1 joue un rôle clef dans cette apoptose induite par l'IL-21. Cette induction d'apoptose pourrait jouer sur l'effet inhibiteur de l'IL-21 dans la production d'IgE. En effet, la vaccination avec *Mycobacterium bovis bacillus* (BCG) induit la production d'IL-21 par les NKT V$\alpha$14 qui, en contre-partie, induit de façon préférentielle l'apoptose des cellules B exprimant des IgE par rapport aux cellules B qui expriment d'autres Igs [85]. Ce mécanisme d'apoptose ciblée et spécifique semble être dû à l'induction par l'IL-21 d'un complexe *Bcl-2/Bcl-2-modifying factor* (Bmf) qui est spécifiquement exprimé chez les cellules B exprimant des IgE. Bmf inhibe l'activité anti-apoptotique de Bcl-2, ce qui induit l'apoptose spécifique des cellules B exprimant des IgE et l'absence conséquente de production d'IgE. Ces données ont permis de montrer que l'IL-21 peut influencer de façon différencielle le devenir d'une réponse médiée par les anticorps selon le type d'activation que les cellules B ont subit lorsqu'elles ont rencontré un antigène. Les cellules B qui ont reçu un signal polyclonal et non spécifique, comme ceux médiés par les TLR, produiront des Igs non spécifiques aux antigènes incluant des auto-anticorps. Au contraire, les cellules B qui interagiront de façon spécifique par l'intermédiaire de leur BCR et qui recevront des signaux provenant des cellules T produiront des anticorps spécifiques.

**L'IL-21 dirige la différenciation terminale des cellules B en plasmocytes:**

Le rôle de l'IL-21 dans la réponse médiée par les anticorps a été étudié en utilisant des souris transgéniques pour l'IL-21 ainsi qu'en leur injectant des plasmides codant pour l'IL-21 [113]. Dans les deux systèmes expérimentaux, l'apoptose induite par l'IL-21 fut observable chez les cellules B naïves *ex vivo*. Cependant, une augmentation du nombre

total de cellules B spléniques a été observée dans les deux modèles expérimentaux. L'augmentation du nombre de cellules B était due à une augmentation du nombre de cellules B immatures, de cellules B *post-switch* et de cellules plasmatiques. Cependant, aucun changement fut observé au niveau des cellules B matures, ces données étant relation avec une augmentation des niveaux sériques d'IgM et d'IgG1 [113]. L'habileté de l'IL-21 à augmenter la différenciation des cellules B en plasmocytes est expliquée par sa capacité à induire l'expression du facteur Blimp-1 (*B lymphocyte-induced maturation protein-1*), un facteur de transcription ayant un rôle clef dans les changements de programe transcriptionnels aboutissant à la différenciation terminale des cellules B en cellules plasmatiques [113, 114]. De façon surprenante, l'IL-21 induit aussi l'expression du facteur de transcription Bcl-6 au niveau des cellules B spléniques ainsi que dans les lignées cellulaires de type B [113]. Les fonctions de Blimp-1 et Bcl-6 sont mutuellement exclusives. Blimp-1 et Bcl-6 se régulent de façon négative l'un l'autre et leurs fonctions et expression corrèlent avec des phénotypes de cellules plasmatiques et de cellules B mémoires respectivement [114, 115]. Il n'est donc toujours pas clair si l'IL-21 induit l'expression de ces deux facteurs dans la même cellule d'un individu. Si c'est le cas, leur co-induction induite par l'IL-21 pourrait être limitée à un stage spécifique du développement des cellules B ayant lieu avant l'étape de dissociation entre cellules mémoires et cellules plasmatiques.

De même que pour les cellules B de souris, l'IL-21 joue un rôle majeur dans la différenciation des cellules B chez l'homme (Figure 7). Les cellules B naïves du sang de cordon ainsi que les cellules B mémoires post-switch peuvent être différenciées en plasmocytes par l'IL-21 lorsque combinée à des signaux provenant du BCR et/ou d'un anti-CD40 [116]. L'IL-21 induit aussi l'expression de Blimp-1 et de l'AID sans induire d'hypermutation somatique [116]. Bien que l'IL-21 permet un switch vers la production d'IgG1 et IgG3 par les cellules B humaines périphériques [117], la co-stimulation de cellules B naïves du sang de cordon avec l'IL-21 et un anti-CD40 induit de façon préférentielle la production d'IgG3 [116]. Ceci suggère que les différences moléculaires de ces populations jouent dans le type d'anticorps produits. Une étude a démontré que la mise en culture de cellules B naïves avec le ligand du CD40 (CD40L) et de l'IL-21 permet aussi la production de cellules B qui produisent des IgA [118]. Cette étude

démontre que dans ces conditions (en présence de CD40L), alors que la présence de l'IL-4 et de l'IL-21 augmente de façon synergique la production d'IgG1, la présence d'IL-4 abolit en parallèle le *switch* vers la production d'IgA [118]. De même, une étude ayant pour but d'évaluer l'utilisation possible de l'IL-21 pour augmenter la production d'Igs chez des patients souffrants d'immunodéficience commune variable ou d'une déficience dans la production d'IgA a démontré que la présence d'IL-21, d'IL-4 et d'anti-CD40, permettait un *switch* de la production d'IgG vers la production d'IgA au niveau des cellules B chez ces patients [119]. De plus, le traitement de ces cellules avec ce cocktail activateur a permis de prévenir l'apoptose spontanée de ces dernières [119].

Bien que l'IL-21 et l'IL-4 coopèrent de façon positive ou négative dans la production d'Igs, ces deux cytokines sont antagonistes dans leur effet sur la différenciation terminale en plasmocytes pour les cellules B murines ou humaines. L'IL-4 inhibe l'effet différenciateur de l'IL-21 chez les cellules B stimulées par des anti-IgM ou des anti-IgM + anti-CD40 alors qu'elle n'inhibe pas son effet sur des cellules B stimulées par des anti-CD40 seuls [113, 116]. L'interation fonctionnelle entre ces deux cytokines est donc complexe et dépend du type de co-stimulation ainsi que de l'état de développement des cellules B.

Bien que les niveaux d'IL-21R soient supérieurs sur les cellules B naïves que les cellules B mémoires [105], l'IL-21 peut induire une différenciation rapide des cellules B mémoires de la zone marginale en plasmocytes [120]. Cette différenciation a lieu en réponse à l'IL-21 et BAFF/BlyS, une cytokine produite par une population de cellules dendritiques locales [121]. La combinaison de BAFF et de l'IL-21 conduit à une induction synergétique de Blimp-1 et de l'AID, entraînant ainsi la différenciation de cellules plasmatiques productrices d'IgG de façon indépendante aux antigènes [120].

La collaboration entre les cytokines concernant la production des anticorps a aussi fait l'objet de plusieurs études. Une étude a démontré que l'IL-6 permettait d'augmenter l'expression de l'IL-21 à partir de cellules T $CD4^+$ naïves et mémoires activées au niveau de leurs TCR [122]. Il semble que cette production d'IL-21 soit nécessaire pour que l'IL-6 médie son rôle promoteur dans la production d'anticorps par les cellules B *in vitro*. Lors d'une étude *in vivo*, l'administration de l'IL-6 et du virus de l'influenza inactivé a permis d'augmenter la production d'anticorps spécifiques au virus et cette

augmentation était dépendante de la présence d'IL-21 [122]. Il semble donc qu'en jouant de façon indirecte sur la production d'IL-21, l'IL-6 augmente la production d'anticorps spécifiques.

Les centres germinaux sont des sites anatomiques dans lesquels les cellules B activées par des antigènes se différencient en cellules plasmatiques pour produire des anticorps de hautes affinités. La différenciation en cellules plasmatiques est régulée par de multiples facteurs dans les environnements créés par les centres germinaux et spécialement les cytokines. Au niveau des centres germinaux, les cellules B interagissent avec les cellules T helper folliculaire (Thf) qui produisent de l'IL-21. Une étude a montré que l'IL-21 permet de mieux initier que l'IL-10 la différenciation des centroblastes ($CD77^{+}$) en cellules plasmatiques en activant STAT-3 et en induisant de façon importante l'expression de facteurs de transcriptions dans les centres germinaux [123]. Cependant, l'IL-10 permet de mieux générer les cellules plasmatiques terminales $CD138^{+}$ à partir des cellules $CD20^{-}CD38^{+}$ au niveau des centres germinaux [123]. Ces différents effets entre l'IL-21 et l'IL-10 sont aussi en relation avec l'expression de leurs récepteurs respectifs aux différents stages. L'IL-21 joue donc un rôle premier et initiateur dans la différentiation des centroblastes alors que l'IL-10 joue un rôle tardif et permet une différenciation terminale des cellules plasmatiques, ce qui permet la production d'Igs [123].

L'étude menée par Linterman et al. démontre que l'IL-21 agit de façon directe sur les cellules B pour contrôler la formation des centres germinaux [124]. L'absence d'IL-21 empêche la maturation des cellules B permettant leur spécificité et réduit le pourcentage de cellules B produisant des IgG1. L'IL-21 peut de plus s'associer aux signaux médiés par le TLR7 pour permettre une réponse optimale des cellules B et la production d'anticorps [125]. Une étude similaire menée par Zotos et al. démontre que l'absence d'IL-21 affecte profondément la réponse des cellules B lors de stimulations avec des antigènes, réduit la formation de cellules plasmatiques dans la rate et la moelle osseuse, et réduit la fonction et la persistence des centres germinaux [126]. Ceci influencera finalement la prolifération des cellules B, leur transition vers le stade mémoire, et réduira leur affinité aux antigènes. Leur étude démontre que cette régulation médiée par l'IL-21 provient de cellules T $CD3^{+}$, probablement des cellules Thf, et que l'IL-21 agit

de façon directe sur les cellules B en maintenant l'expression de Bcl-6 [126]. Il semble donc que l'IL-21 contrôle le devenir des cellules B en agissant de façon directe sur ces cellules au niveau des centres germinaux.

### 2-3-2 Effet de l'IL-21 sur les cellules T CD4$^{+}$

**L'IL-21 est produite par de multiples populations de cellules T helper:**

Chacune des sous-populations de cellules T (Th1, Th2 et Th17) peuvent produire de l'IL-21 ainsi que répondre à cette dernière bien que leur réponse soit différente. Cependant, l'implication des sous-types de cellules T responsables de la production d'IL-21 aux différentes étapes d'une réponse immunitaire reste inconnue.

Les études menées pour savoir quel type de cellule T helper produit l'IL-21 ont été contreversées et inconsistantes dans le passé [127, 128]. Une étude a montrée que l'IL-21 était produite par les cellules Th2 et non les Th1 alors qu'une autre étude utilisant des puces à ADN avait démontré le contraire [127, 128]. Des études récentes ont démontré que les cellules Th17 produisaient des niveaux élevés d'IL-21 par rapport aux Th1 et Th2 [129, 130]. L'IL-21 elle-même, ainsi que l'IL-6, sont essentielles dans cette augmentation d'expression dans ces cellules [129-131]. La signalisation médiée par le TCR des Th17 semble aussi augmenter l'expression de l'IL-21 par ces dernières.

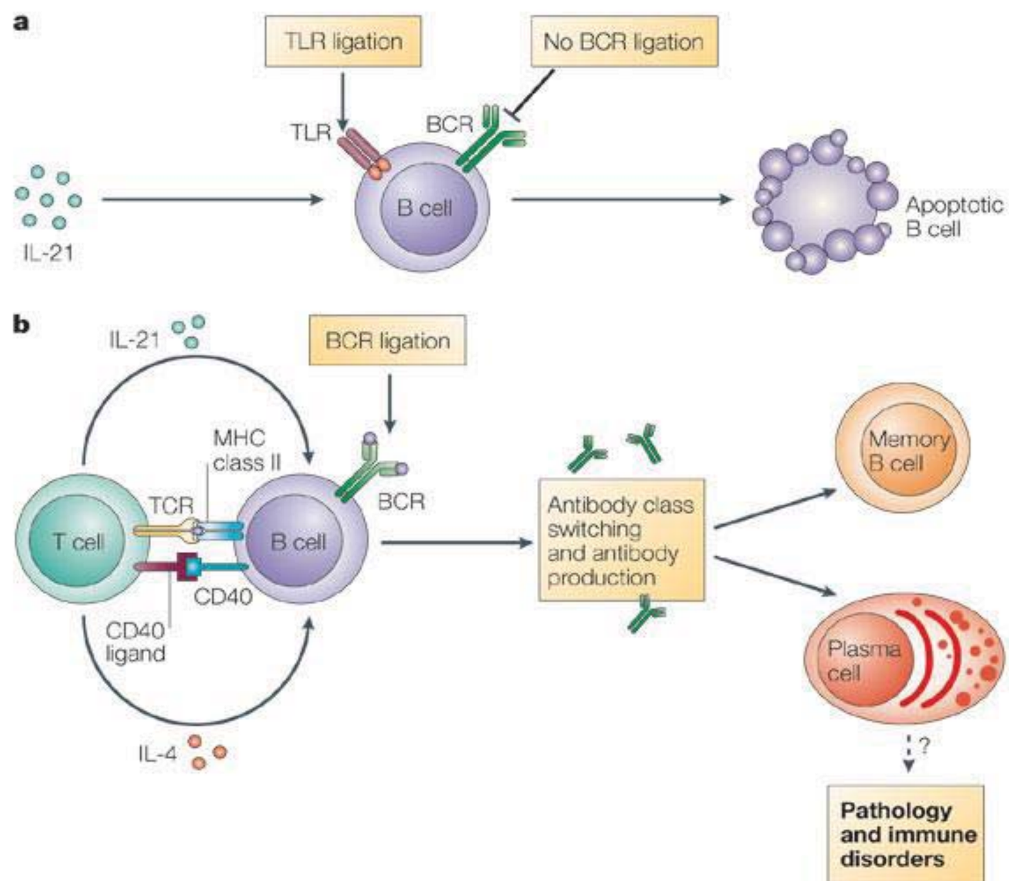
**Effets *in vitro* de l'IL-21 sur la différentiation Th1 versus Th2:**

De façon analogue aux effets de l'IL-21 sur les cellules B, l'effet de l'IL-21 sur cette différenciation dépend d'autres cytokines ou cellules immunitaires ainsi que le stade de développement des cellules T (Figure 8). L'observation initiale suggérant que l'IL-21 fonctionnait comme une cytokine de type Th2 est basée sur des expériences *in vitro* qui ont montré sa capacité à inhiber l'expression d'IFN-γ chez des cellules T CD4$^{+}$ naïves cultivées dans des conditions menant à un phénotype Th1 [127]. Il n'y avait cependant pas de sous-régulation générale du programme Th1 car le traitement par l'IL-21 n'a eu aucun effet sur l'expression de T-bet ou du IL-12Rβ2, qui sont des marqueurs de type Th1 exprimés en absence d'IL-21. La réduction spécifique d'IFN-γ par l'IL-21 est

directement reliée à la répression de l'Eomesodermine, un facteur de transcription de type T-box qui joue un rôle important dans l'induction d'IFN-γ (Figure 8) [132]. De plus, le traitement avec l'IL-21 de cellules T de sang périphérique pré-activées au niveau de leur TCR induit l'expression d'une panel de gènes de type Th1 comme ceux codant pour l'IFN-γ, T-bet et l'IL-12Rβ2, ce qui suggère un effet différent de l'IL-21 sur des cellules T déjà activées par rapport à des cellules naïves [133].

**Effet *in vivo* de l'IL-21 sur la différenciation Th1 versus Th2:**

Les souris KO pour l'IL-21R ont un profil normal de développement des cellules T $CD4^+$ tant au niveau du thymus qu'en périphérie [103]. De plus, la stimulation *in vitro* de cellules T $CD4^+$ naïves vers un profil Th1 ou Th2 n'a montré aucune différence dans l'expression d'IFN-γ ou d'IL-4 chez des souris sauvages ou KO pour l'IL-21R. Ceci suggère que l'IL-21 n'est pas essentielle pour la différenciation de ces deux sous-types de cellules T [103]. Lorsque les souris KO pour l'IL-21R ont été examinée dans un modèle retardé d'hypersensibilité, les souris KO ont montré une réponse imflammatoire élevée par rapport aux souris sauvages. Le challenge *ex vivo* par un antigène spécifique des cellules T $CD4^+$ a révélé une haute production d'IFN-γ par les souris KO par rapport aux souris contrôles [127]. Une autre étude a examiné l'expression de l'IL-21 lors de l'infection par *Schistosoma mansoni*, un parasite reconnu pour induire un réponse de type Th2 [134]. Les niveaux d'IL-21 ont été mesurés dans des souches de souris développant une réponse massive de type Th1 (double KO pour l'IL-4 et l'IL-10) ou de type Th2 (double KO pour l'IL-12 et l'IL-10). Bien que l'infection ait induit une réponse hautement polarisée dans les poumons avec la production d'IL-13 et d'IFN-γ, l'IL-21 a été produite lors de l'infection par les deux types de souris, indiquant que l'IL-21 ne se comporte pas comme une cytokine classique de type Th1 ou Th2 [134]. Les souris KO pour l'IL-21R infectées par *Schistosoma mansoni* n'ont pas eu d'augmentation dans la production de cytokines de type Th1 malgré une réponse réduite de celles de type Th2 (baisse de la production d'IL-4, d'IL-10 et d'IL-13) au niveau des poumons. De plus, une résolution de l'infection a été plus rapidement observée chez les souris KO pour l'IL-21R, ce qui suggère un rôle joué par l'IL-21 dans l'initiation et la maintenance de la réponse inflammatoire.

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*(Leonard et al. Nat Rev Immunol. 2005)*

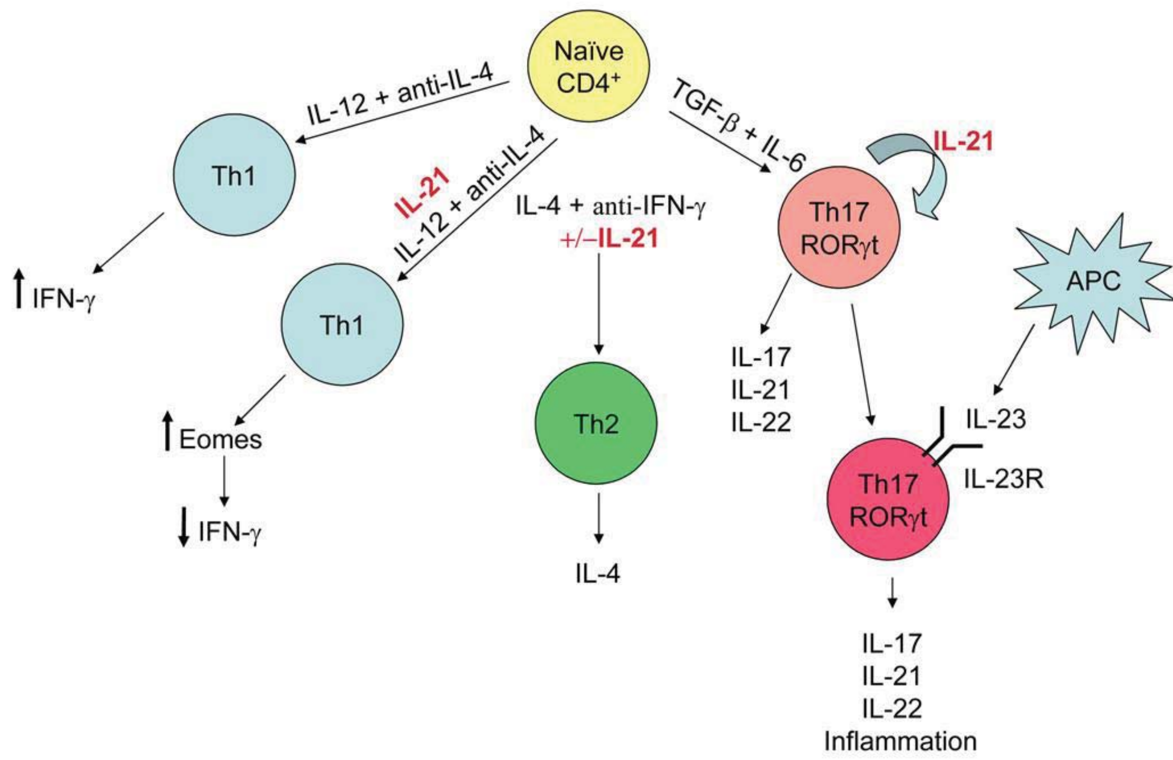
**Figure 7. Effet de l'IL-21 sur les cellules B**

Lors de cette étude, aucune différence dans le profil d'expression des cytokines par des cellules T $CD4^+$ stimulées *ex vivo* avec des antigènes a été observée, ce qui suggère que la déficience *in vivo* de l'IL-21 n'altère en rien le *priming* des cellules T $CD4^+$ vers un sous-type précis, et va plutôt conduire à une baisse de différenciation vers une réponse de type Th2.

Lors d'une autre étude, des souris KO pour l'IL-21R infectées par un parasite intestinal (*Heligmosomoides polygyrus*) qui conduit à des fièvres et des petits granulomes, ont subi une réduction d'éosinophylie dans le sang, ce qui suggère un défaut de la réponse Th2 [135]. Cependant, l'analyse de la production cytokinique *ex vivo* des souris infectées a révélé aucune différence dans les niveaux d'expression d'IL-4 et d'IFN-γ.

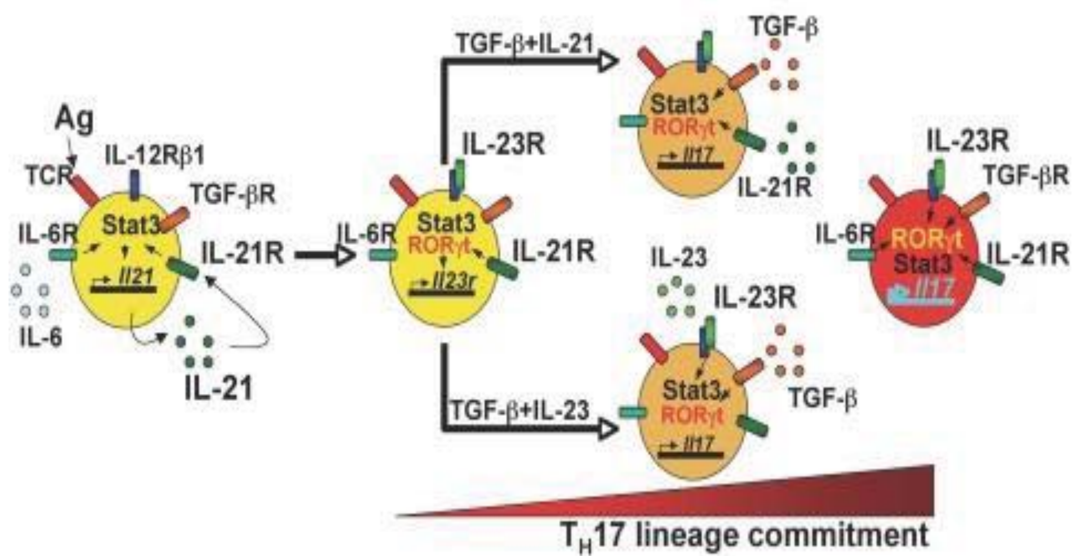
### 2-3-3 Effet de l'IL-21 sur les cellules Th17

Les cellules T $CD4^+$ qui produisent de l'IL-17 possèdent des distinctions développementales et fonctionelles par rapport aux cellules Th1 ou Th2 (Figure 9) [136, 137]. Le TGF-β, qui joue un rôle clef dans la génération des cellules T régulatrices (Trég), possède aussi un rôle important dans l'induction et la différenciation des cellules Th17 [138-140]. Le TGF-β régule d'une façon fine le développement des Trég et des Th17, notamment grâce à l'IL-6 qui permet de balancer la différenciation en faveur du développement des cellules Th17 et réduit celle des Trég [141]. Une analyse à partir de cellules T $CD4^+$ stimulées au niveau de leur TCR a démontré que l'IL-6 induisait fortement l'expression de l'IL-21 [131]. En effet, l'IL-21 est très fortement exprimée dans les cellules Th17 et semble être approximativement cinq fois plus exprimée dans ces cellules que dans les cellules Th1 et Th2 (Figure 8 et 9) [129, 130]. L'induction de l'IL-21 conduit à sa propre augmentation de façon autocrine [131]. L'IL-21 induit l'expression de l'IL-23R sur ces cellules [129, 131]. L'IL-23R forme un dimère avec l'IL-12Rβ1 qui est partagé par l'IL-12R [142]. Bien que l'IL-23 soit un facteur important dans les maladies inflammatoires tant chez l'homme que chez la souris, son récepteur n'est pas présent sur les cellules T naïves mais l'IL-23 joue un rôle important dans l'expansion et la maintenance des cellules Th17 (Figure 9) [143].




***(Spolski et al. Curr Opin Immunol. 2008 )***

**Figure 8. Effet de l'IL-21 sur les cellules T**




*(Leonard et al. J Leuk Biol. 2008 )*

**Figure 9. Effet de l'IL-21 sur le développement des cellules Th17**

L'induction de l'IL-23R par l'IL-21 à partir de cellules T CD4$^+$ naïves est par conséquent une étape critique dans la différenciation et l'expansion des cellules Th17 *in vivo* (Figure 9). L'IL-21 et l'IL-23 augmentent l'expression du facteur de transcription nécessaire au développement de ces cellules, le RORγt, qui conduit lui-même à la trancription de l'IL-21 [131, 144]. La production d'IL-17 est réduite chez les cellules T CD4$^+$ de souris KO pour l'IL-21R après différenciation *in vitro* avec de l'IL-6 et du TGF-β, ce qui démontre que l'induction de l'IL-21 par l'IL-6 conduit à une amplification de la différenciation [129, 131]. En fait, l'IL-23R n'est pas induit à la surface des cellules T CD4$^+$ lors d'une différenciation *in vitro* avec de l'IL-6 et du TGF-β chez des souris KO pour l'IL-21 ou l'IL-21R, ce qui indique un rôle critique de l'IL-21 dans le contrôle de l'expression de l'IL-23R [129, 131]. L'induction initiale de l'IL-21 par les cellules Th17 est donc critique pour établir une amplification de type autocrine qui conduira à la différenciation terminale, l'expansion, la maintenance et la production d'IL-17 par ces dernières (Figure 9).

L'étude menée par Huber et al. a démontré le rôle clef de *l'Interferon Regulatory Factor 4* (IRF-4) dans l'induction des cellules Th17 par l'IL-21 [145]. Lors de cette étude, un profond défaut dans la génération des cellules Th17 a été observé en présence d'IL-21 et de TGF-β à partir de cellules de souris KO pour l'IRF-4. Les niveaux de l'IL-23R étaient réduit ainsi que ceux des facteurs de transcription RORγt et RORα alors que l'expression du facteur de transcription FoxP3 (classique des cellules Trég) était augmentée [145]. Une autre étude a démontré que les souris KO pour la protéine IBP (*IRF-4 Binding Protein*), une protéine reconnue pour inhiber l'action de l'IRF-4, développent rapidement de l'arthrite rhumatoïde et des vasculites [146]. Il semble que ces signes d'inflammation soient dûs à une augmentation de la réponse des cellules T et à une synthèse inapropriée d'IL-17 et d'IL-21. L'IBP empêchait en effet la régulation des gènes codant pour l'IL-17 et l'IL-21 en séquestrant l'IRF-4. Il semble donc que l'IBP joue un rôle important dans la prévention contre les maladies auto-immunes médiées par les cellules T en régulant la production d'IL-17 et d'IL-21 en réponse aux antigènes du soi.

### 2-3-4 Effet de l'IL-21 sur l'induction des cellules T régulatrices

L'IL-21 joue un rôle indirect sur la différenciation des cellules T régulatrices (Trég) qui sont impliquées dans la suppression des réponses immunitaires. L'IL-21, contrairement à l'IL-2, ne supporte pas la prolifération des cellules Trég activées par un anti-CD3 [147, 148]. L'IL-21 semble réduire l'expression du facteur de transcription FoxP3 qui est nécessaire pour le développement de ces cellules bien que le mécanisme exact ne soit pas toujours clair. L'effet négatif de l'IL-21 sur ces cellules est de plus démontré par la présence de quatre à cinq fois plus de cellules T $CD4^+$ $FoxP3^+$ chez les souris KO pour l'IL-21 [129].

### 2-3-5 Effet de l'IL-21 sur les lymphocytes T $CD8^+$

Les souris KO pour l'IL-21 ne démontrent aucun problème concernant le développement de ces cellules [103]. Plusieurs expériences *in vitro* ont démontré que l'IL-21 a un faible effet sur les cellules T $CD8^+$ naïves et mémoires mais peut augmenter leur prolifération de façon synergétique avec l'IL-7 ou l'IL-15, deux cytokines reconnues pour avoir un rôle majeur dans l'expansion de ces cellules [149-151]. Cet effet synergétique est observé lorsque les cellules T $CD8^+$ sont stimulées en absence d'activation du TCR, ce qui suggère un rôle de l'IL-21 dans une expansion de ces cellules qui est antigène-indépendante [149]. Des études utilisant des *micro-array* ont démontré que l'IL-21 augmentait l'expression de l'ARN messagé codant pour le granzyme B, qui à un rôle dans la réponse cytotoxique, et le facteur c-jun qui joue un rôle dans le contrôle de la prolifération cellulaire [149]. L'IL-21 est aussi reconnue pour augmenter l'expression de la granulysine dans ces cellules, une protéine ayant un rôle important dans l'activité anti-microbienne et dans le contrôle d'infections opportunistes pouvant se déclarer chez des patients atteints du SIDA ou souffrant d'immunodéficiences diverses (Figure 10) [152].

Les cellules T $CD8^+$ effectrices sont reconnues pour avoir une faible expression des molécules co-stimulatrices CD28 et CD62L. Des études ont démontré que l'IL-21

permettait d'empêcher la sous-régulation de CD28 et CD62L et servait donc à maintenir une co-stimulation importante au niveau de ces cellules [153]. Bien que l'IL-21 peut sous-réguler l'expression de CD44 sur les cellules T $CD8^+$, l'effet combinatoire de l'IL-15 avec l'IL-21 augmente l'accumulation de cellules T $CD44^{high}CD8^+$ [149]. De même, une combinaison de l'IL-21 avec l'IL-15 permet d'augmenter l'expression d'IFN-γ par ces cellules T $CD8^+$ [149]. Lors d'études *in vivo*, une immunisation avec un virus de la vaccine exprimant la glycoprotéine 160 (gp160) du VIH-1 chez des souris KO pour l'IL-21R a entraîné une baisse dans la prolifération et la cytotoxicité des cellules T $CD8^+$ par rapport aux souris sauvages [149]. Ceci suggère que l'IL-21 à un rôle dans l'expansion et la différenciation fonctionnelle des cellules T $CD8^+$ qui sont antigènes-spécifiques. Lorsque des cellules T $CD8^+$ naïves sont stimulées *in vitro* par des cellules dendritiques matures présentant un peptide tumoral, une augmentation dans la prolifération de ces cellules a été observée lors de l'ajout d'IL-21, entraînant une accumulation de cellules T $CD8^+$ cytotoxiques caractérisées par un haut niveau d'expression de CD28, une affinité antigènique multipliée par 10, et une augmentation de leur capacité à sécréter de l'IL-2 [154]. L'IL-21 possède la capacité d'augmenter la prolifération antigène-indépendante des cellules T $CD8^+$ naïves et mémoires. Elle peut aussi augmenter la prolifération et la différenciation antigène-dépendante des cellules T $CD8^+$ naïves mais non celle des mémoires. La base de cette différence entre ces deux sous-types de cellules T $CD8^+$ n'est pas encore comprise. Une étude sur les cellules T $CD8^+$ effectrices chez des patients infectés par le VIH-1 a révélée que l'IL-21 augmentait la production de perforine en absence d'activation ou de prolifération, alors que l'activation de perforine par l'IL-15 était moins importante et était dépendante d'une prolifération cellulaire [155]. L'induction de perforine par l'IL-21 dans les cellules T mémoires était plus importante chez les patients infectés que les patients non infectés. Ainsi, l'effet prolifératif et fonctionnel de l'IL-21 sur les cellules T $CD8^+$ diffère au niveau des cellules naïves et des cellules mémoires [155].

Une accumulation des cellules T $CD8^+$ $CD28^-$ s'accumule avec l'âge, ceci étant dû à des stimulations antigèniques répétées avec le temps et aux effets homéostatiques régulés par des cytokines comme l'IL-15. Une étude récente réalisée par Nguyen et Weng a démontré que l'IL-21 permet de renforcer l'effet de l'IL-15 en ce qui concerne l'entrée

dans le cycle cellulaire des cellules T CD8$^+$ [156]. L'IL-21 augmentait préférentiellement la prolifération des cellules T CD8$^+$ CD28$^+$ mémoires par rapport aux cellules T CD8$^+$ CD28$^-$ car les cellules CD28$^+$ exprimaient de plus haut niveau d'IL-15R et d'IL-21R et présentaient une phosphorylation plus importante de STAT-5 après stimulation. De plus, l'IL-21 a réduit la sous-régulation de CD28 induite par l'IL-15 dans les cellules T CD8$^+$ mémoires. La capacité proliférative et de survie en réponse à une stimulation combinée entre ces deux cytokines fut bien maintenue avec l'âge. Cette étude a donc démontré que l'IL-21 augmentait la prolifération des cellules T CD8$^+$ mémoires, particulièrement les cellules CD28$^+$, et que cette cytokine pourrait servir d'antagoniste à l'effet réducteur de l'IL-15 concernant l'expression du CD28 sur ces cellules avec le temps [156].

### 2-3-6 Effet de l'IL-21 dans la génération des cellules Th folliculaires

L'IL-21 est de nos jours reconnue pour avoir un rôle important dans la génération des cellules T helper folliculaires (Thf) [157, 158]. Ces cellules T sont retrouvées au niveau des tissus et spécialement au niveau des centre germinatifs où elles vont collaborer avec les cellules B et permettre leur différenciation finale conduisant à la production d'anticorps de haute affinité [124]. Les cellules T helper folliculaires sont de phénotype CD4$^+$CXCR5$^+$ et expriment le facteur de transciption Bcl-6. Ces cellules produisent des hauts niveaux d'IL-21 qui vont pouvoir agir directement sur les cellules B présentent au niveau des centres germinatifs [124, 126].

Il semble que la molécule ICOS ait un rôle clef dans la génération des cellules Thf et la régulation de l'expression du facteur de transcription c-Maf qui à son tour régule l'expression de l'IL-21 [80]. Les souris KO pour ICOS n'ont pas de défaut dans la différenciation des cellules Th17 mais ont un faible nombre de ces dernières ainsi que peu de cellules Thf après stimulation avec de l'IL-23. Les cellules Thf présentes chez les souris KO pour ICOS expriment peu d'IL-17. Ces études ont démontré que la stimulation par ICOS active c-Maf qui va à son tour augmenter l'expression de l'IL-21 dans ces deux types cellulaires et ainsi réguler leur expansion [80]. Plusieurs autres

études démontrent aussi l'importance de l'interaction entre les cellules Thf et les cellules B à travers la molécule ICOS concernant le développement des centres germinatifs. Cette interaction va conduire à la production d'IL-21 par les cellules Thf et permettre la formation de ces centres [159, 160].

Une étude a aussi démontré que l'IL-12 produite par les cellules dendritiques humaines permettait aux cellules T $CD4^+$ naïves de devenir des cellules de type Thf produisant de l'IL-21 [161]. Ces cellules T de type Thf auront de plus la capacité d'aider les cellules B à produire des anticorps en sécrétant de l'IL-21 et en les stimulants par la voie utilisant ICOS. Cette étude suggère donc une co-opération des cytokines et spécialement l'IL-12 produite par les cellules dendritiques au niveau des tissus concernant la différenciation des cellules Thf. De ce fait, une dérégulation dans les fonctions de ces cellules aboutissant à une dérégulation dans la production d'IL-21 pourrait créer des troubles dans la production d'anticorps ou le développement de maladies auto-immunes [162].

### 2-3-7 Effet de l'IL-21 sur les cellules Natural Killer

Le développement des cellules NK dépend de la présence des cytokines $\gamma_c$ dépendantes [163, 164]. Cependant, les souris KO pour l'IL-21R ont une nombre normal de cellules NK fonctionnelles, ce qui suggère que l'IL-21 ne possède pas de rôle clef dans le développement de ces cellules [103, 165]. L'IL-21 peut par contre jouer un rôle dans la maturation et le développement fonctionnel de ces cellules à un niveau tardif du développement. Une étude a en effet démontré l'effet de l'IL-21 dans la génération *in vitro* des cellules NK à partir de précurseurs provenant de moelle osseuse [73]. Bien que les souris KO pour la $\gamma_c$ n'ont pas de cellules NK matures, les précurseurs NK de la moelle osseuse ($CD122^+$ $NK1.1^-$ $CD49b^-$) se developpent même en absence de signaux $\gamma_c$-dépendants. Seulement un petit sous-type de ces précurseurs expriment le récepteur à l'IL-21 [166]. L'augmentation de l'expression de l'IL-21R au niveau de ces cellules est dépendante de l'IL-15, ce qui suggère que l'IL-15 augmente la sensibilité des précurseurs NK aux signaux médiés par l'IL-21 [166]. Une étude utilisant des précurseurs NK provenant de sang de cordon (cellules $CD34^+$) a démontré que ces

cellules auront une meilleure différenciation si de l'IL-21 est ajouté au milieu de culutre permettant leur développement, qui est constitué d'IL-15, de Flt3-L (*FMS-like tyrosine kinase 3 ligand*), de facteurs de cellules souches et de l'IL-7 [167].

Une autre étude a été réalisée sur la différenciation des cellules NK à partir de cellules du sang de cordon ombilical CD34$^{-}$ en présence de Flt3-L, de SCF (*Stem Cell Factor*) et des cytokines IL-15 et IL-21 seules ou en combinaison [168]. Leur étude démontre qu'en présence d'IL-15 et d'IL-21, les cellules CD34$^{-}$ prolifèrent et vont acquérir une morphologie lymphoïde ainsi qu'exprimer un phénotype KIR$^{+}$ et CD56$^{+}$/CD16$^{+/-}$ correspondant à un phénotype de cellules NK pseudo-matures. De plus, ces cellules produisent de l'IFN-$\gamma$, du GM-CSF, du CCL3/MIP-1$\alpha$ et sont capable d'exprimer en surface le marqueur CD107a après contact avec une cellule cible.

L'IL-21 augmente la prolifération des cellules NK immatures en combinaison avec l'IL-2 ou l'IL-15 alors qu'elle n'a aucun effet si utilisée seule [169]. L'IL-21 a un effet bi-phasique sur les cellules NK immatures (Figure 10). En effet, de faible doses d'IL-21 augmentent leur prolifération alors que de grandes doses l'inhibent, même en présence d'IL-2 ou d'IL-15.

L'IL-21 possède aussi un effet au niveau de la prolifération, de la survie et au niveau de l'expression de certains récepteurs chez les cellules NK matures. L'IL-21 possède aussi une effet négatif concernant leur prolifération lorsqu'elles ont été stimulées par de l'IL-15 et peut induire leur apoptose (Figure 10) [170].

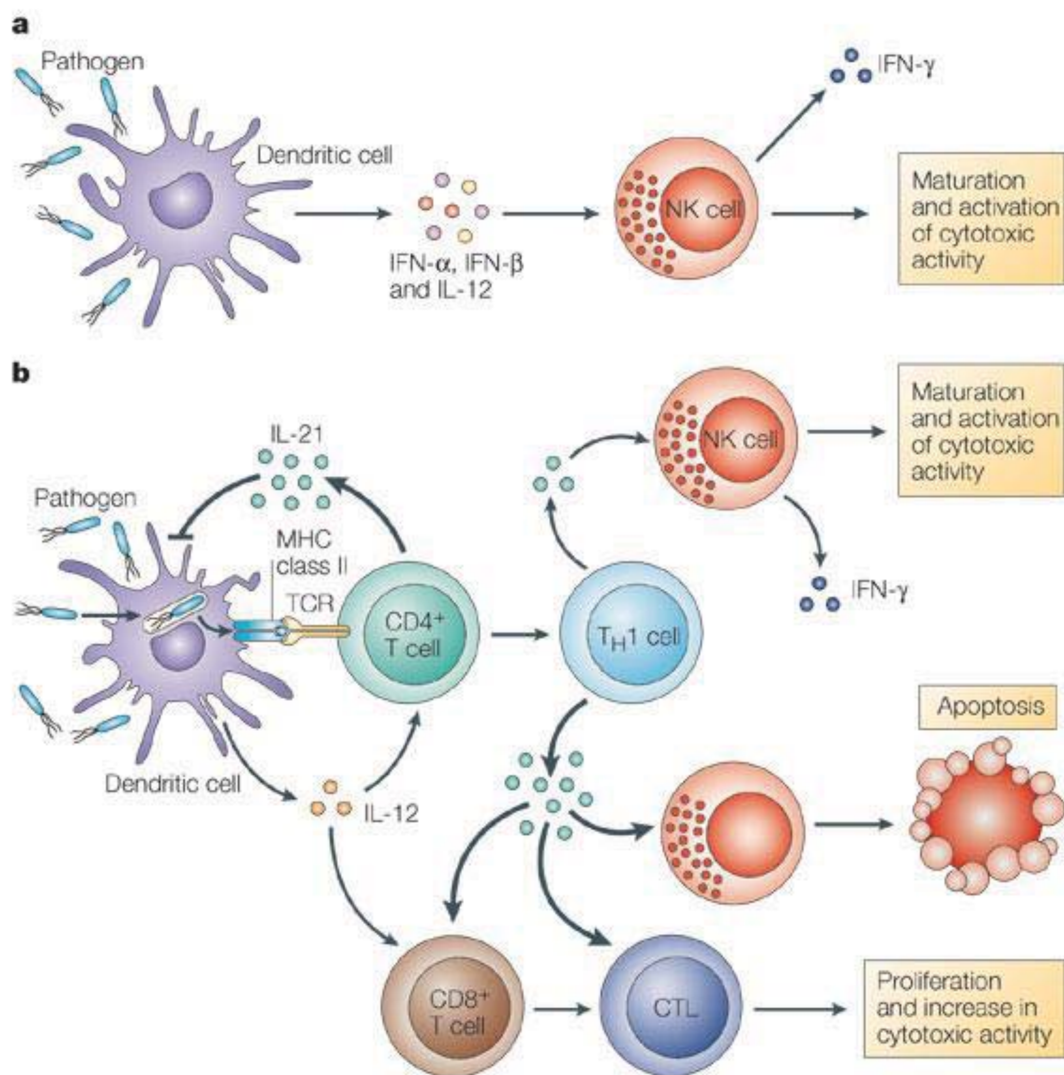
L'IL-21 augmente leur activité cytotoxique et la production d'IFN-$\gamma$ (Figure 10) [165, 170]. Contrairement à l'IL-2 et l'IL-15 qui augmentent l'expression des récepteurs inhibiteurs murins Ly49, l'IL-21 inhibe ces derniers à leurs surfaces [171]. De même, la signalisation médiée par le récepteur NKG2D, qui est dépendante de son partenaire de signalisation DAP-10, est inhibée par l'IL-21 car cette cytokine sous-régule ce facteur au niveau transcriptionnel [172]. Cependant, l'IL-21 augmente l'expression de NKG2D à la surface des cellules NK murines dérivées de moelle osseuse, ce qui suggère encore une fois un rôle de l'IL-21 qui est spécifique au stage de développement des cellules NK [170].

### 2-3-8 Effet de l'IL-21 sur les cellules dendritiques

L'ensemble des études portant sur l'interaction entre l'IL-21 et les cellules dendritiques (CD) ont démontré que cette cytokine inhibe leur maturation et leurs fonctions (Figure 10). Une des première étude portant sur ce sujet a démontré que l'injection d'un plasmide codant pour l'IL-21 dans des souris a entraîné une augmentation en périphérie de cellules positives pour CD11b et Gr1, deux marqueurs spécifiques à ces cellules [173]. La génération *in vitro* de cellules dendritiques en présence d'IL-21 aboutit à des cellules possédant des phénotypes immatures caractérisés par un faible niveau d'expression du complexe majeur d'histocompatibilité de classe II (CMH-II), une haute capacité à capturer des antigènes et un faible niveau d'expression du récepteur aux chimiokines CCR7 [97]. Lorsque ces CD ont été traitées avec du lypopolysaccharide (LPS), qui est supposé terminer leur maturation, aucune augmentation des niveaux d'expression du CMH-II, du CD86 et du CD80 (qui sont des marqueurs de maturité) n'a été observé [97]. De plus, ces cellules avaient une faible capacité à promouvoir la prolifération des cellules T lors de co-cultures. Le traitement de ces CD pendant seulement deux heures avec de l'IL-21 et un antigène ovalbumine, suivi de leur transfert adoptif *in vivo*, leur a permis d'inhiber un contact d'hypersensibilité médié par les cellules T.

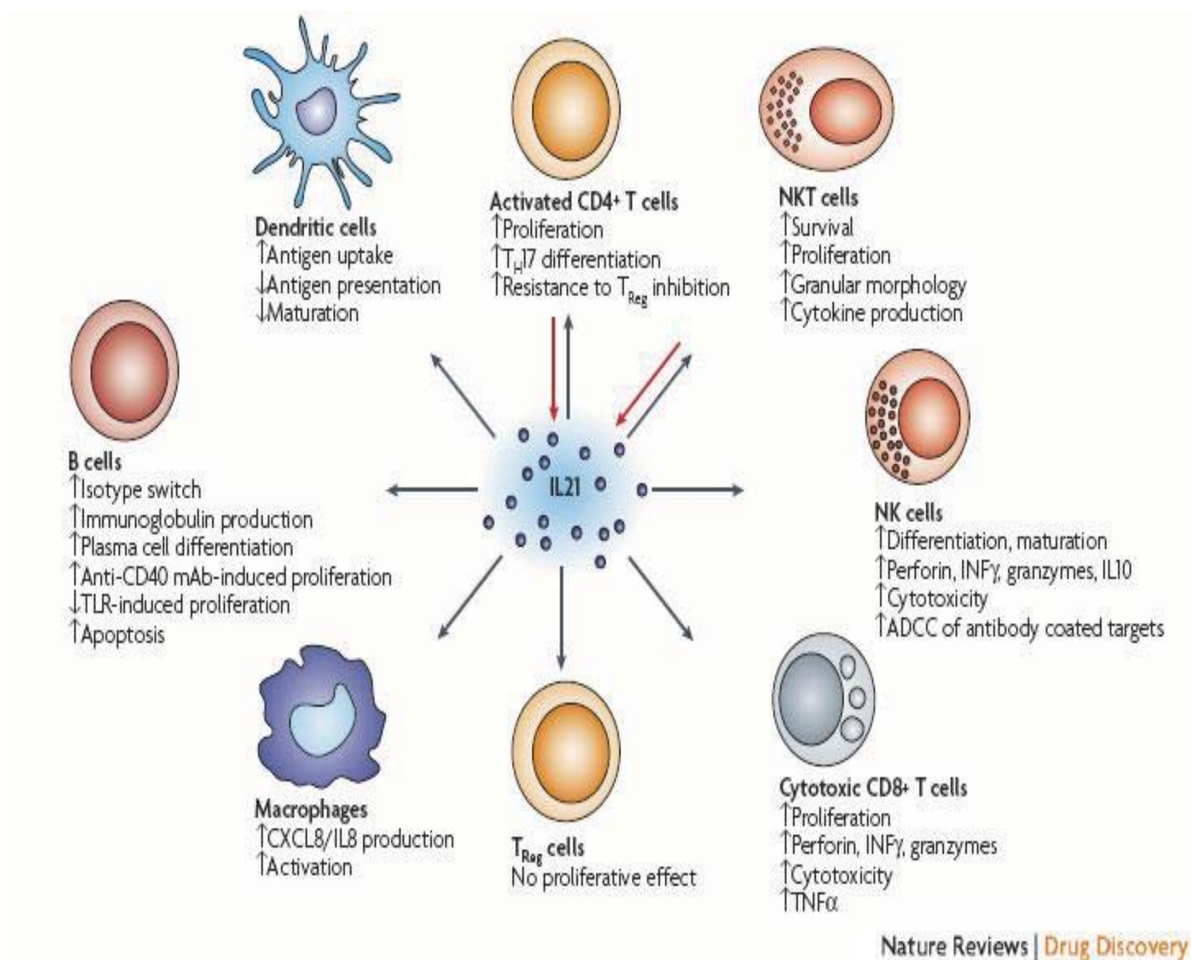
### 2-3-9 Effet de l'IL-21 sur les cellules NKT

Les cellules NKT sont une population de cellules T qui expriment un répertoire du TCR restreint. Elles reconnaissent des glycolipides présentés par le CD1d et possèdent des récepteurs activateurs et inhibiteurs présents chez les cellules NK [174]. Elles régulent les autres cellules en produisant des cytokines et possèdent une activité cytotoxique importante. L'IL-21 augmente leur prolifération après activation avec un anti-CD3 et de l'IL-21 ou de l'IL-15 et stimule aussi la relâche d'IL-4 et d'IL-13 par ces cellules [77].




*(Leonard et al. Nat Rev Immunol. 2005)*

**Figure 10. Effets de l'IL-21 sur la réponse immunitaire médiée par les cellules T et les cellules Natural Killer**




*(Skak et al. Nat Rev Drug Discov. 2008)*

**Figure 11. Schéma résumant les différents effets de l'IL-21 sur les cellules immunes**

L'IL-21 induit l'expression de granzyme B et induit des changements morphologiques qui rendent ces cellules similaires au cellules NK (larges et granulaires) (Figure 11). Les cellules NKT produisent de l'IL-21 lorsqu'elles sont stimulées *in vitro* avec un anti-CD3 ou *in vivo* avec de l'α-GalCer, un glycolipide spécifique aux cellules NKT (Figure 11) [77]. Les niveaux d'IL-21 produits par les NKT sont plus élevés que ceux produits par les cellules T $CD4^+$ spléniques lors d'une stimulation avec un anti-CD3. Cette capacité à produire de hauts niveaux d'IL-21 en réponse à un stimuli microbien peut suggérer que ces cellules pourraient réguler les étapes initiales permettant la mise en place d'une réponse immune acquise médiée par les lymphocytes T et B.

### 2-3-10 Effet de l'IL-21 sur les macrophages

L'IL-21 possède un effet pro-inflammatoire indirect sur les macrophages en induisant la production du facteur CXCL8 (*CXC-chemokine ligand 8*, autrement appelé IL-8) (Figure 11) [175]. Ce facteur chimio-attracteur va attirer les neutrophiles et leur permettrent de passer de la circulation sanguine à travers les vaisseaux endothéliaux vasculaires pour arriver au niveau des tissus inflammés. Bien que les neutrophiles n'expriment pas l'IL-21R, l'IL-21 peut donc, de façon indirecte, jouer un rôle inflammatoire et permettre aux macrophages de recruter des neutrophiles ou d'autres cellules immunes dans les tissus inflammés.

De plus, le rôle critique joué par l'IL-21 dans le développement des cellules Th17 conduit indirectement à la production de cytokines appartenant à la famille de l'IL-17, qui affectent le recrutement et les fonctions des neutrophiles [129-131]. Ainsi, l'IL-21 possède la capacité de freiner ou moduler de façon fine la réponse immune des cellules présentatrices d'antigènes professionnelles.

L'étude menée par Rückert et al. a démontré que des souris KO pour l'IL-21R possèdent un nombre normal de macrophages, ce qui suggère un rôle non essentiel de l'IL-21 dans la génération de ces cellules [176]. Leur étude démontre que les macrophages matures expriment l'IL-21R et la chaîne commune γ. Cependant, une stimulation courte avec de l'IL-21 n'augmente pas leur prolifération mais induit l'expression de protéines

régulatrices du cycle cellulaire et anti-apoptotiques p21 et p27, ainsi que l'expression des protéines SOCS2 et SOCS3 (*Suppressor of Cytokine Signaling 2 et 3*) [176].
De plus, l'IL-21 augmente la phagocytose des macrophages et l'activité de la métalloprotéinase de matrice 12 (MMP-12). La stimulation des macrophages avec l'IL-21 augmente leur capacité à induire la prolifération des cellules T CD4$^+$ antigène-spécifiques [176]. Cependant, aucune augmentation de la prolifération a été observée concernant les cellules T CD8$^+$ antigène-spécifiques.

## 2-4 Rôle de l'IL-21 dans l'auto-immunité et les maladies inflammatoires

Plusieurs études on proposé un rôle important de l'IL-21 dans le développement de maladies auto-immunes ou inflammatoires (Figure 12).

### 2-4-1 Rôle de l'IL-21 dans les maladies auto-immunes

La première observation qui a suggéré un rôle potentiel de l'IL-21 dans une maladie auto-immune médiée par les cellules B a été réalisée à partir d'un model murin de Lupus Systémique Erythématoseux (LSE), BXSB.B6-*Yaa*$^+$ [113]. En effet, le développement de la maladie était en corrélation avec les concentrations d'IL-21 dans les sérums. Ceci était consistant avec les niveaux élevés d'IgG observés et le rôle de l'IL-21 dans la différenciation des plasmocytes [113]. Les souris BXSB-*Yaa* KO pour l'IL-21R ne développaient pas les signes caractéristiques du Lupus, comme l'hypergammaglobulinémie, la production d'auto-anticorps, une fréquence réduite des cellules B de la zone marginale et des problèmes rénaux, par rapport aux souris sauvages [177]. Il semble de plus que l'IL-21 causant cette auto-immunité ne provenait ni des cellules Th17 ou des cellules Thf mais des cellules T CD4$^+$ ICOS$^+$ spléniques. Des études sur un modèle murin de Lupus MRL-Fas/lpr ont montré que les cellules T CD4$^+$ de ces souris produisent des haut niveaux d'IL-21, comme c'est les cas des souris *Yaa* [178, 179]. Le traitement de ces souris avec un récepteur à l'IL-21 soluble et couplé à une partie Fc (IL-21R-Fc) a permis de réduire la lympho-adénopathie, de réduire les

changements morphologiques des glomérules du rein et de réduire légérement les niveaux d'IgG1 et d'IgG2a [178, 179].

L'étude menée par Webb et al. démontre que l'expression de l'IL-21R sur les cellules B de patients atteints de LSE est réduite et que les niveaux sériques d'IL-21 sont augmentés chez ces patients, comme c'est le cas pour les modèles murins de cette maladie [180]. De plus, le poylmorphisme existant au niveau du gène codant pour l'IL-21 ainsi que son récépteur est associé avec le développement de cette maladie [180, 181].

L'étude menée par Liu et al. démontre dans un modèle d'uvéite auto-immune expérimentale (EAU), que l'ARN messagé codant pour l'IL-21 est fortement exprimé dans les noeuds lymphatiques et la rate par rapport aux souris contrôles [182]. L'expression de l'IL-21R est aussi augmentée dans ces différents compartiments.

Une autre observation a été réalisée avec les souris mutantes sanroque, qui ont un défaut d'expression de la roquine, une protéine qui régule négativement la production des cellules T folliculaires (Thf) qui produisent de l'IL-21. Ces souris ont donc des niveaux élevés de cellules Thf ainsi que des niveaux élevés d'IL-21 circulantes, ce qui en contre-partie augmente les niveaux d'anticorps anti-nucléaires, de glomérulonéphrites et des lympho-adénopathie périphériques [183]. L'hypothèse émise serait que les niveaux élevés d'IL-21 conduiraient à une augmentation d'anticorps auto-réactifs par les cellules B folliculaires.

Les études sur des souris NOD (*Non Obese Diabetic*) ont démontré que leurs cellules T produisaient des niveaux élevés d'ARN codant pour l'IL-21 et ont suggéré que ces haut niveaux pourraient augmenter la prolifération de cellules T $CD8^+$ auto-réactives qui détruisent les cellules β du pancréas [184]. Une autre étude a proposé la possibilité que le gène codant pour l'IL-21, appartenant au locus *Idd3* (*Insulin-dependent diabetes susceptibility 3 locus*), est un déterminant génétique qui pourrait prédisposer les personnes au développement du diabète [185]. Les souris NOD KO pour l'IL-21R sont en effet résistantes à l'inflammation, à la production d'auto-anticorps contre l'insuline et résistante au développement du diabète de type 1 [186, 187]. Chez ces souris, on a observé un défaut prononcé dans la présence de cellules T auto-réactives lors d'expériences de transferts. De plus, l'étude menée par cette équipe démontre que la sur-

expression d'IL-21 dans les cellules β du pancréas induit la production de cytokines et chimiokines inflammatoires incluant l'IL-17A, l'IL-17F, l'IFN-γ, le MCP-1 et 2 et l'IP-10. L'infiltration des leukocytes dans les ilôts pancréatiques subséquemment à cette inflammation a entraîné la destruction des cellules β et un diabète spontané de type 1 [187]. L'étude de McGuire et al. a démontré que cette grande production d'IL-21 résultait de la présence de deux SNPs (*Single Nucletodide Polymorphism*) dans la région promotrice distale du gène codant pour l'IL-21, ce qui réulte sur une plus haute affinité pour cette région à la liaison du facteur de trancription Sp1 [188].

Certaines études proposent aussi un rôle de l'IL-21 et de l'IL-15 dans le développement de la maladie de Coeliac, une entéropathie causée par une intolérance au glutène chez des individus génétiquement susceptibles. Il semblerait que les cellules immunes présentes au niveau de l'intestin de ces patients produiraient ces deux cytokines qui orchestreraient en contre-partie une inflammation mucosale et engendrerait cette maladie [189].

Les études cliniques récentes de phase II concernant l'utilisation de l'anticorps monoclonal alemtuzumab (Campath-1H) contre la sclérose multiple ont permis d'observer que l'IL-21 avait un rôle déterminant dans le développement d'une auto-immunité secondaire [190]. En effet, 30% des patients traités par cet anticorps ont développé une auto-immunité secondaire des mois voire des années après le traitement. Or, les patients qui ont développé une auto-immunité secondaire avaient, avant le traitement par cet anticorps, des niveaux d'IL-21 sériques plus élevés que ceux qui n'ont pas développés d'auto-immunité secondaire [190]. Les auteurs de cette étude suggèrent donc que l'IL-21 serait un biomarqueur de risque concernant le développement d'une seconde auto-immunité après le traitement par cet anticorps. Les niveaux élevés d'IL-21 permettrait l'expansion des cellules T qui vont les conduire à une apoptose excessive, ce qui augmentera au final leurs chances de rencontrer des auto-antigènes et finalement promouvoir une auto-immunité [190]. Cependant, il semble que l'IL-21 n'ait pas de rôle majeur dans le développement des encéphalites auto-immunes et des myocardites auto-immunes [191, 192].

### 2-4-2 Rôle de l'IL-21 dans les maladies inflammatoires

L'IL-21 augmente l'encéphalite allergique expérimentale (EAE), un modèle expérimental pour la sclérose multiple, qui peut être induite par injection d'un antigène de myéline en présence d'adjuvants [193]. Lorsque l'IL-21 est injectée avant l'induction de la maladie, la sévérité de la maladie est augmentée et peut être mesurée par l'augmentation de cellules inflammatoires dans le système nerveux central. Cependant, si l'IL-21 est injectée après l'induction de la maladie, aucun effet n'est observé sur la maladie [193]. Ce phénomène semble être de plus dépendant des cellules NK car une déplétion de ces cellules avant l'induction de la maladie empêche l'augmentation de la sévérité médiée par l'IL-21. Bien que les cellules NK semblent être impliquées dans ce phénomène, une étude a démontré que l'IL-21 jouait un rôle dans l'induction et l'expansion des cellules Th17 dans ce modèle d'EAE [129, 130]. Les souris KO pour l'IL-21 ou l'IL-21R possèdent une réduction de dix fois du nombre de cellules produisant de l'IL-17 et de la progression de la maladie, comme c'est le cas des souris KO pour l'IL-17 [129, 130]. Les souris KO pour l'IL-21 ont aussi un nombre élevé de cellules T régulatrices (Trég). Ainsi, l'augmentation des symptômes auto-immuns chez les souris injectées avec l'IL-21 avant l'induction de l'EAE pourrait augmenter le nombre de cellules Th17 et réduire celui des Trég, ce qui augmenterait la sévérité de la maladie [130]. Lors d'une étude, l'utilisation d'agent bloquant les effets biologiques de l'IL-21 a permis d'exacerber le développement de l'EAE, d'augmenter la production d'IL-17 ainsi que de réduire le nombre et l'activité des cellules Trég [194]. Cependant, les mécanismes contrôlant ces résultats contreversés sont inconnus.

Une étude portant sur l'arthrite rhumatoïde (AR) a démontré que l'expression de l'IL-21 corrèle avec la présence des cellules Th17 au niveau du fluide synoviale et du sang périphérique des patients atteints de la maladie [195]. En utilisant un modèle murin d'AR, Jang et al. ont montré que les souris KO pour l'IL-21R sont totalement réfractaires au développement de cette maladie [196]. Ces souris contiennent moins de cellules T $CD4^+$ ayant des capacités prolifératives, un nombre réduit de cellules Thf et un nombre plus élevé de cellules Th17 que les souris sauvages. Leurs travaux

démontrent que ces souris ne développent pas de cellules B mémoires produisant des IgG1 qui sont spécifiques aux auto-antigènes. De plus, l'IL-21 a induit l'expression de la protéine RANKL (*Receptor Activator of NF-κB Ligand*), un régulateur de l'ostéoclastogénèse [196]. Peu de cellules infiltrantes exprimant RANKL ont été découvertes dans le fluide synovial des souris KO pour l'IL-21R. Il semble donc que l'IL-21 réalise un rétro-contrôle positif autocrine impliquant l'homéostasie des cellules T $CD4^+$ et spécialement celle des cellules Thf. Le nombre réduit de cellules Thf jouerait sur un défaut de maturation des cellules B et le développement de cellules B auto-réactives, permettant ainsi le développement de l'arthrite rhumatoïde [196]. Dans un modèle d'arthrite induite par le collagène, un inhibiteur de l'IL-21 (IL-21R-Fc) a permis de réduire faiblement l'inflammation [179]. Andersson et al. ont analysé l'effet bloquant de l'IL-21R-Fc et d'un anti-IL-15 sur la production de cytokines pro-inflammatoires au niveau des cultures cellulaires de membranes synoviales provenant de patients atteints d'AR [197]. Le fait de bloquer ces deux cytokines, et plus spécialement l'IL-21, a permis de réduire la production d'IL-1β, d'IL-6 et de TNF-α de façon très significative, suggérant un rôle joué par ces cytokines dans cette inflammation.

Une étude a aussi démontré une expression augmentée de l'IL-21 en analysant des biopsies rectales de patients atteints de colites ulcératives [198]. De même, la présence de variant génétiques et du polymorphisme dans la région contenant l'IL-2 et l'IL-21 a été associée au développement de la colite ulcérative [199].

L'étude menée par Caruso et al. a démontré que l'IL-21 était fortement exprimée dans la peau des patients souffrant de psoriasis [200]. Cette cytokine stimule de plus la prolifération des kératinocytes et cause une hyperplasie épidermale lorsqu'on injecte cette dernière de façon intra-dermale dans des souris. En utilisant un modèle murin dans lequel le psoriasis est xénogreffé, la neutralisation de l'IL-21 a permis d'abolir l'inflammation et de réduire la prolifération des kératinocytes [200].

Une étude a démontré que la proportion des cellules T $CD4^+$ produisant de l'IL-21 dans la *lamina propria* intestinale est augmentée chez les patients atteints de maladies

inflammatoires de l'intestin par rapport aux patients sains [201]. Ces cellules expriment aussi de l'IFN-γ et à un moinde niveau de l'IL-4 et de l'IL-17A. L'activation de ces cellules par de l'IL-12 et non de l'IL-23 a d'ailleurs permis d'augmenter la fréquence des cellules doubles positives pour l'IL-21 et l'IFN-γ. Dans cette étude, les auteurs ont aussi montré que la fréquence des cellules Thf produisant de l'IL-21 était plus élevée chez les patents atteins de la maladie de Crohn que ceux atteints de colite ulcérative et des personnes saines. Le traitement de ces cellules Thf avec de l'IL-12 a encore une fois permis d'augmenter la production d'IL-21 dans ces cellules. Ceci suggère qu'au niveau de la *lamina propria* intestinale, les cellules produisant de l'IL-21 produisent aussi de l'IFN-γ [201].

Ainsi, les études provenant de plusieurs laboratoires à travers le monde ont documenté de façon claire l'effet bénéfique que pouvait avoir l'inhibition de l'IL-21 sur la progression de maladies inflammatoires et auto-immunes.

### 2-5 Rôle de l'IL-21 dans l'allergie et l'asthme

La capacité de l'IL-21 à sous-réguler la production d'IgE a permis aux scientifiques de proposer un rôle pour l'IL-21 dans la diminution de la sévérité des allergies et de l'asthme (Figure 12). En effet, les études réalisées sur des modèles murin de rhinite allergique ont montré que l'administration d'IL-21 réduisait de façon significative les symptômes allergiques. Les souris traitées avec l'IL-21 possédaient des niveaux sériques plus faibles d'IgE spécifiques aux antigènes et des concentrations réduites de cytokines de type Th2 (IL-13, IL-4, IL-5) dans les tissus nasaux [202]. De plus, ces souris possédaient des niveaux faibles d'éotaxine-1 et-2 (induits par l'IL-4) dans les fibroblastes nasaux, ce qui a conduit à une suppression de la migration des éosinophiles dans les tissus nasaux.

De façon similaire, l'administration systémique d'IL-21 dans un modèle murin d'allergie alimentaire a permis de bloquer une anaphylaxie induite par les antigènes [203]. Cette

inhibition était accompagnée par l'induction d'Id2 (un répresseur de la recombinaison vers la production d'IgE) et une diminution correspondante d'IgE spécifiques aux antigènes. La relation entre la prévention de la production d'IgE par l'IL-21 et l'anaphylaxie a été prouvée en montrant que l'IL-21 ne pouvait pas bloquer l'anaphylaxie chez des souris qui était KO pour l'Id2 [203].

Cependant, les souris KO pour l'IL-21R utilisées dans un modèle d'inflammation généralisée ont présenté de façon innatendue moins d'hyper-réponses antigène-induites des voies inflammatoires par rapport aux souris contrôles, malgré le fait d'avoir des hauts niveaux sériques d'IgE spécifiques aux antigènes [135]. Ceci suggère que l'activation des mastocytes par les IgE ne représente seulement qu'un seul des mécanismes impliquant les cytokines dans ce type d'inflammation.

Une étude récente a mis en relation le rôle de l'IL-21R et le développement de la dermatite atopique (DA), une maladie inflammatoire allergique commune de la peau due aux allergènes. L'expression de l'IL-21 et de l'IL-21R était augmentée au niveaux de lésions de peau chez les patients atteint de DA [204]. De même, leur expression était augmentée au niveau du modèle murin correspondant à cette maladie. De plus, les souris KO pour l'IL-21R aini que les souris sauvages du modèle traitées par un inhibiteur de l'IL-21 (IL-21R-Fc) n'ont pas développé d'inflammation après une sensibilisation de leur peau de façon mécanique. Il semble que les souris KO pour l'IL-21R avaient une migration réduite des cellules dendritiques de la peau vers les noeuds lymphatiques, ce qui a entraîné une faible acccumulation de cellules T $CD4^+$ au niveau de ces organes lymphoïdes secondaires [204]. De plus, leurs cellules dendritiques (IL-21R KO) n'ont pas subit d'augmentation de l'expression de CCR7 et ne migraient pas en réponse aux ligands de ce récepteur. En effet, il semble que le traitement de cellules dermales et épidermales avec l'IL-21 a augmenté l'expression de la métalloprotéinase de matrice MMP-2, qui est impliquée dans la migration des cellules dendritiques de la peau.

### 2-6 Rôle de l'IL-21 dans la réponse anti-tumorale

La capacité de l'IL-21 à réguler la réponse immune médiée par les cellules T ainsi que les cellules NK a suggéré un possible effet anti-tumoral pour cette dernière. L'effet anti-tumoral de l'IL-21 a été examinée dans de nombreux modèles murins de tumeurs [205, 206]. L'ensemble de cet effet anti-tumoral est associé à l'activation de la réponse immune médiée par les cellules T $CD8^+$ et les cellules NK. Les différents modèles tumoraux qui ont été étudiés sont présentés dans un article de revue publié et présenté en tant qu'article numéro 1. Cet article comprend des informations plus détaillées sur l'effet anti-tumoral de l'IL-21 ainsi que sur les essais cliniques en cours impliquant cette cytokine. Cet article a été soumis à un processus de révision par les pairs avant sa publication.

**Article 1** : **Iannello A** and Ahmad A. Interleukin-21, new adjuvant for cancer therapy ? 2009. ***Médecine Sciences*** **25(4): 341-3**.

**Résumé:** L'interleukine-21 (IL-21) est une interleukine récemment découverte faisant partie de la famille des cytokines dont la signalisation intracellulaire est dépendante de la chaîne commune γ après interaction avec leurs récepteurs comme l'IL-2, 4, 7, 9 et 15. Cette cytokine est principalement produite chez la souris comme chez l'humain par les lymphocytes T $CD4^+$ activés et les cellules TH17. Elle joue un rôle multifonctionnel et pléïotropique sur le système immunitaire. La compréhension du rôle des cytokines dans le système immunitaire, ainsi que leur répercussion sur le développement des cancers, a amené les chercheurs à essayer de bloquer ces dernières comme c'est le cas pour l'IL-6 ou à les utiliser en tant qu'agents thérapeutiques dans le cas de l'IL-2. Récemment, de nombreuses études portant sur la biologie de l'IL-21 ont permis d'envisager des études précliniques portant sur l'effet de l'IL-21 sur les tumeurs. Ces études précliniques convaincantes réalisées sur des modèles murins ont permis aux scientifiques de commencer des essais cliniques chez l'homme utilisant l'IL-21 seule ou en combinaison avec d'autres agents thérapeutiques pour le traitement des cancers.

# <u>Article 1</u>

**Iannello A** and Ahmad A. Interleukin-21, new adjuvant for cancer therapy ? 2009. ***Médecine Sciences*** **25(4): 341-3**.

le cerveau et présente donc un intérêt particulier, puisque pour la première fois des altérations du gène codant pour une protéine intervenant dans la voie de signalisation de l'IGF-1 sont associées directement à une maladie neuro-dégénérative. L'étude approfondie de l'impact de ces variations géniques sur la fonction de la protéine nous apportera de précieuses informations sur son mode d'action et sur le croisement des voies métaboliques et neurologiques. ◊

**Glucose homeostasis, diabetes and neurodegenerative disorders**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### RÉFÉRENCES


1. Plum L, Belgardt BF, Bruning JC. Central insulin action in energy and glucose homeostasis. *J Clin Invest* 2006 ; 116 : 1761-6.
2. Zhao WQ, Alkon DL. Role of insulin receptor in learning and memory. *Mol Cell Endocrinol* 2001 ; 177 : 125-34.
3. Craft S, Watson GS. Insulin and neurodegenerative disease: shared and specific mechanisms. *Lancet Neurol* 2004 ; 3 : 169-78.
4. Yasuda H, Terada M, Maeda K, *et al.* Diabetic neuropathy and nerve regeneration. *Prog Neurobiol* 2003 ; 69 : 229-85.
5. Abuzzahab MJ, Schneider A, Goddard A, *et al.* Igf-I receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med* 2003 ; 349 : 2211-22.
6. Ristow M. Neurodegenerative disorders associated with diabetes mellitus. *J Mol Med* 2004 ; 82 : 510-29.
7. Peila R, Rodriguez BI, Launer LJ. Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: The Honolulu-Asia aging study. *Diabetes* 2002 ; 51 : 1256-62.
8. Qiu WQ, Walsh DM, Ye Z, *et al.* Insulin degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. *J Biol Chem* 1998 ; 273 : 32730-8.
9. Farris W, Mansourian S, Chang Y, *et al.* Insulin degrading enzyme regulates the levels of insulin, amyloid β-protein, and β-amyloid precursor protein intracellular domain *in vivo*. *Proc Natl Acad Sci USA* 2003 ; 100 : 4162-7.
10. Hu G, Jousilahti P, Bidel S, *et al.* Type 2 diabetes and the risk of Parkinson's disease. *Diabetes Care* 2007 ; 30 : 842-7.
11. Dufresne AM, Smith RJ. The adapter protein Grb10 is an endogenous negative regulator of insulin-like growth factor signaling. *Endocrinology* 2005 ; 146 : 4399-409.
12. Giovannone B, Lee E, Laviola L, *et al.* Two novel proteins that are linked to insulin-like growth factor (Igf-I) receptors by the Grb10 adapter and modulate Igf-I signaling. *J Biol Chem* 2003 ; 278 : 31564-73.
13. Yoshihashi H, Maeyama K, Kosaki R, *et al.* Imprinting of human Grb10 and its mutations in two patients with Russell-Silver syndrome. *Am J Hum Genet* 2000 ; 67 : 476-82.
14. Lautier C, Goldwurm S, Durr A, *et al.* Mutations in the Gigyf2 (Tnrc15) gene at the Park11 locus in familial Parkinson d isease. *Am J Hum Genet* 2008 ; 82 : 822-33.
15. Helmer C, Pasquier F, Dartigues JF. Épidémiologie de la maladie d'Alzheimer et des syndromes apparentés. *Med Sci (Paris)* 2006 ; 22 : 288-96.
16. Checler F, Alves da Costa C, Dumanchin-Njock C, *et al.* Métabolisme du précurseur du peptide amyloïde et présénilines. *Med Sci (Paris)* 2002 ; 18 : 717-22.


### GLOSSAIRE

**RI :** Récepteur de l'insuline
**R-IGF-1R :** Récepteur de l'IGF-1
**GIGYF2 :** GRB10 interacting GYF protein 2
**Grb10 :** Growth factor receptor-bound protein 10
**IRS :** Insulin receptor substrate
**PI3K :** Phosphatidylinositol 3 kinase
**PKC :** Protein kinase C
**PDK :** Phosphoinositide-dependent kinase
**PKB :** Protein kinase between kinase A and C
**GSK-3β :** Glycogen Synthase Kinase 3β
**SHC :** Src homologous and collagen protein
**Grb2 :** Growth factor receptor binding protein 2
**NGF :** Nerve growth factor
**BDNF :** Brain derived neurotrophic factor
**NT :** Neurotrophin,
**Trk :** Tyrosine receptor kinase
p75 NTR, p75 neurotrophin receptor

**NOUVELLE**

# L'interleukine-21, un nouvel adjuvant contre le cancer ?


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> L'interleukine-21 (IL-21) est une interleukine récemment découverte faisant partie de la famille des cytokines. Le récepteur de l'IL-21 est un hétérodimère entre IL-21R et la chaîne commune γ, cette dernière étant également partagée par les récepteurs de plusieurs interleukines, IL-2, 4, 7, 9 et 15. L'IL-21 est principalement produite, chez la souris comme chez l'humain, par les lymphocytes T CD4+ activés et les cellules TH17. Elle a de multipltes fonctions sur le système immunitaire (*Figure 1*) [1]. Le rôle des cytokines dans le système immunitaire, et leur répercussion sur le développement des cancers ont amené les chercheurs à essayer d'en bloquer l'action, comme c'est le cas pour l'IL-6 [2], ou à les utiliser en tant qu'agents thérapeutiques dans le cas de l'IL-2 [3]. Récemment, de nombreuses études portant sur la biologie de l'IL-21 et des études précliniques convaincantes réalisées sur des modèles murins ont permis aux scientifiques de commencer des essais cliniques chez l'homme utilisant l'IL-21 seule ou en combinaison avec d'autres agents thérapeutiques pour le traitement des cancers.

## Études précliniques utilisant l'IL-21

Ses effets sur la stimulation des cellules *Natural Killer* (NK) et des lymphocytes T CD8+ (LTCD8+), justifient l'utilisation depuis peu de temps de l'IL-21 à des fins thérapeutiques contre différents types de cancers. En effet, de nombreux groupes ont montré dans des études indépendantes que la transfection de




Nouvelles.indd 16 25/03/2009 10:53:41

l'ADNc codant pour l'IL-21 dans des cellules tumorales murines (issues de cancer du côlon, de mélanome, de fibrosarcome, de cancer mammaire, de cancer de la vessie, de gliome, de carcinome rénal et de neuroblastome) aboutit à une faible croissance ou au rejet total de ces tumeurs [4]. De plus, une stratégie vaccinale, *via* l'injection de cellules tumorales exprimant l'IL-21 et irradiées dans un modèle murin d'adénocarcinome mammaire, a démontré un prolongement de la survie de ces souris [5]. Des résultats identiques sur la réduction de la croissance tumorale et la survie des souris ont été obtenus dans les modèles B16 de mélanome ou de fibrosarcome, en présence d'un plasmide codant pour l'IL-21 [7]. La présence d'IL-21 favorise aussi le transfert adoptif de LTCD8+ tumoraux spécifiques dans le modèle murin de mélanome B16, retardant la croissance de la tumeur avant son élimination [6], ou dans le modèle murin de thymome EG.7 [7].

| Phases | Traitement | Types de cancers | Résultats |
|---|---|---|---|
| **Essais achevés** | | | |
| Phase I | IL-21 seule | Mélanome métastatique (29 patients) | 1 réponse partielle<br>9 états stables<br>15 progressions<br>4 sans évaluation |
| Phase I/II | IL-21 seule | Mélanome métastatique (24 patients) | 1 réponse complète<br>4 réponses partielles |
| | | Carcinome à cellule rénale (19 patients) | 24 états stables<br>14 progressions |
| Phase I/II | IL-21 et Rituximab | Lymphome B (15 patients) | 2 réponses complètes<br>3 réponses partielles<br>8 états stables |
| **Essais en cours** | | | |
| ***IL-21 seule*** | | | |
| Phase I | IL-21 seule | Mélanome métastatique<br>Carcinome à cellule rénale | En attente |
| Phase I | IL-21 seule | Mélanome métastatique | En attente |
| Phase I | IL-21 seule | Mélanome | En attente |
| ***IL-21 en combinaison*** | | | |
| Phase I/II | IL-21 + Sorafenib | Carcinome à cellule rénale | En attente |
| Phase I/II | IL-21 + Sunitinib | Carcinome à cellule rénale | En attente |
| Phase I | IL-21 + Cetuximab | Carcinome de côlon métastatique | En attente |
| Phase I | IL-21 + Doxorubicine | Cancer de l'ovaire | En attente |

***Tableau I. Essais cliniques achevés et en cours concernant l'IL-21.*** Le Sorafenib et le Sunitinib sont des inhibiteurs « multicibles » des récepteurs à tyrosine kinase administrés par voie orale. La doxorubicine est un agent intercalant l'ADN qui inhibe la topo-isomérase II.

### L'IL-21 seule ou en combinaison ?

L'utilisation de l'IL-21 combinée avec des anticorps dirigés contre des cibles tumorales fait également l'objet d'études. La présence d'IL-21 associée au Trastuzumab (anti-HER2, *human epidermal growth factor receptor*) pour le cancer du sein, au Rituximab (anti-CD20, *cluster of differentiation 20*) pour les lymphomes ou au Cetuximab (anti-récepteur à l'EGF, *epithelial growth factor)* pour le cancer du côlon entraîne une augmentation de la cytotoxicité cellulaire dépendante d'anticorps (ADCC : *antibody dependent cell cytotoxicity*) accompagnée par la sécrétion d'IFN-$\gamma$ (*interferon-$\gamma$*) et de TNF-$\alpha$ (*tumor necrosis factor-$\alpha$*) [9]. *In vivo*, le traitement de souris inoculées avec des cellules cancéreuses murines surexprimant le récepteur humain HER2 avec de l'IL-21 associée à l'anticorps 4D5 (anti-HER2 humain) a permis de ralentir la croissance de la tumeur [10]. Des résultats similaires ont été obtenus avec l'association Cetuximab et l'IL-21 dans un modèle expérimental utilisant des cibles tumorales surexprimant le récepteur à l'EGF [10].

Non seulement l'IL-21 active la réponse anti-tumorale relayée par les cellules NK et les LTCD8+, mais, de plus, elle peut aussi avoir un effet anti-tumoral direct sur les cellules cancéreuses de type lymphocytaire. L'IL-21 induit l'apoptose de cellules de leucémie lymphocytaire chronique (LLC) et augmente l'efficacité de l'ADCC en présence du Rituximab [11]. Cette cytokine provoque l'apoptose de cellules provenant de lymphomes B non-hodgkiniens (LCBNH), réduit la prolifération de cellules provenant de lymphomes diffus à grandes cellules B (LDGCB) et déclenche l'apoptose des cellules primaires provenant de lymphomes de types LLC et LDGCB [12, 13]. Cependant, chez les patients atteints de Sézary (forme généralisée d'une prolifération lymphoïde T cutanée), l'IL-21 n'entraîne pas l'apoptose de cellules T provenant de lymphomes cutanés ni celle des cellules malignes circulantes [14, 15].

Nouvelles.indd 17 25/03/2009 10:53:41

## Des essais cliniques prometteurs

Ces études précliniques convaincantes ont stimulé le développement d'essais cliniques chez l'homme *(Tableau 1)*. À ce jour, trois essais cliniques de phase I/II ont été réalisés en Australie et aux États-Unis. Les deux premiers ont porté sur le mélanome métastatique et le « carcinome à cellules rénales ». Le troisième essai clinique a associé IL-21 et Rituximab dans le lymphome B non hodgkinien. L'étude de phase I dans le mélanome métastatique a inclus 29 patients, dont 25 ont pu être évalués : une stabilisation de la maladie a été obtenue chez 9 d'entre eux, et une réponse partielle caractérisée par une baisse de la taille et de la progression des tumeurs chez un patient. Le deuxième essai clinique a porté sur 24 patients atteints de mélanome métastatique et 19 ayant un carcinome à cellules rénales. Le traitement a induit une réponse complète chez les patients atteints de mélanome et 4 réponses partielles dans les cas de cancer du rein. Dans la troisième étude, l'association de l'IL-21 et du Rituximab chez 15 patients ayant des rechutes de lymphomes B a permis d'obtenir deux réponses complètes caractérisées par une disparition totale des tumeurs dans l'organisme, 3 réponses partielles, ainsi que 8 stabilisations.

De nouveaux essais cliniques sont en cours avec l'IL-21 seule ou en combinaison avec d'autres agents *(Tableau 1)*. Deux cytokines, l'IL-2 et l'IFN-α, sont déjà utilisées dans le traitement du mélanome métastatique et du carcinome à cellules rénales, mais elles induisent une réponse antitumorale faible et l'IL-2 s'avère toxique à long terme lorsqu'elle est utilisée aux doses efficaces sur le plan tumoral. Le traitement par l'IL-2 entraîne en effet, un syndrome hémorragique vasculaire qui produit des oedèmes interstitiaux et provoque la défaillance d'organes comme les poumons et le cœur. L'IL-21 est quant à elle considérablement moins toxique que l'IL-2 pour une efficacité anti-tumorale au moins équivalente sinon supérieure, permettant donc d'envisager son utilisation seule ou associée à d'autres agents thérapeutiques, si ces premières données se confirment dans d'autres essais cliniques.

L'IL-21 semble donc à ce jour être la seule cytokine dotée de propriétés antitumorales efficaces, notamment celle d'inhiber la croissance des cellules B malignes, et dont la toxicité est acceptable, aucun effet secondaire mortel ou sévère n'ayant été rapporté à ce jour. Récemment, notre groupe de recherche a démontré un taux faible d'IL-21 circulante chez les patients infectés par le VIH (virus de l'immunodéficience humaine) développant un SIDA (syndrome d'immunodéficience acquise), et une corrélation positive entre l'absence d'IL-21 et la progression vers un SIDA [16]. De plus, nos donnés expérimentales attestent un effet bénéfique de l'IL-21 sur la réponse antivirale relayée par les cellules NK et les $LTCD8^+$. Cette cytokine représente donc l'un des agents immunothérapeutiques les plus prometteurs et les essais cliniques en cours apporteront de précieuses informations sur son efficacité dans le traitement de patients atteints de cancer ou de pathologies associées à des virus ou à des bactéries. ◊

**Interleukin-21, new adjuvant for cancer therapy?**

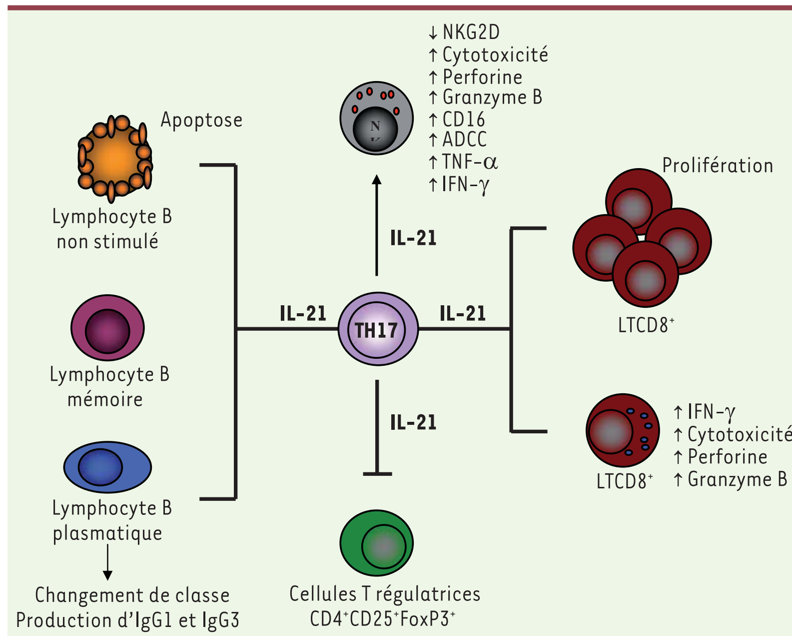


***Figure 1. Effets majeurs de l'IL-21 sur les cellules du système immunitaire.*** L'IL-21 est sécrétée principalement par les lymphocytes T $CD4^+$ activés et les cellules TH17. Elle entraîne la maturation des cellules NK et augmente leur activité cytotoxique contre les cellules tumorales. L'IL-21 augmente la prolifération des lymphocytes T $CD8^+$ ($LTCD8^+$) ainsi que leur activité cytotoxique contre leurs cibles. L'IL-21 inhibe l'activité des cellules T régulatrices dont un des rôles est de contenir la réponse anti-tumorale et spécialement la prolifération des $LTCD8^+$. En l'absence de stimulation, l'IL-21 provoque l'apoptose des lymphocytes B. Cependant, cette cytokine permet la différenciation de cellules B naïves en lymphocytes B mémoires et cellules plasmatiques après une interaction avec un lymphocyte T et une stimulation qui fait intervenir CD40 et son ligand CD40L. L'IL-21 peut aussi promouvoir le changement de classe des immunoglobulines - anticorps et augmenter la production d'anticorps de type IgG1 et IgG3 qui permettent l'opsonisation des cellules cancéreuses. Cette opsonisation entraîne leur élimination par les macrophages et favorise le fonctionnement du mécanisme d'ADCC médié par les cellules *natural killer.*



Nouvelles.indd 18 25/03/2009 10:53:41

## RÉFÉRENCES


1. Spolski R, Leonard WJ. Interleukin-21 : basic biology and implications for cancer and autoimmunity. *Ann Rev Immunol* 2008 ; 26 : 57-79.
2. Gougelet A, Mansuy A, Blay J. De l'eau au moulin de thérapies ciblant l'interleukine-6 dans les tumeurs épithéliales. *Med Sci (Paris)* 2008 ; 24 : 694-6.
3. Kirkwood JM, Tarhini AA, Panelli MC, *et al.* Next generation of immunotherapy for melanoma. *J Clin Oncol* 2008 ; 26 : 3445-55.
4. Andorsky DJ, Timmerman JM. Interleukin-21 : biology and application to cancer therapy. *Exp Opin Biol Ther* 2008 ; 8 : 1295-307.
5. Comes A, Rosso O, Orengo AM, *et al.* CD25+ regulatory T cell depletion augments immunotherapy of micrometastases by an IL-21-secreting cellular vaccine. *J Immunol* 2006 ; 176 : 1750-8.
6. Zeng R, Spolski R, Finkelstein SE, *et al.* Synergy of IL-21 and IL-15 in regulating CD8+ T cell expansion and function. *J Exp Med* 2005 ; 201 : 139-48.
7. Wang G, Tschoi M, Spolski R, *et al. In vivo* antitumor activity of interleukin 21 mediated by natural killer cells. *Cancer Res* 2003 ; 63 : 9016-22.
8. Moroz A, Eppolito C, Li Q, *et al.* IL-21 enhances and sustains CD8+ T cell responses to achieve durable tumor immunity : Comparative evaluation of IL-2, IL-15, and IL-21. *J Immunol* 2004 ; 173 : 900-9.
9. Skak K, Kragh M, Hausman D, *et al.* Interleukin 21 : combination strategies for cancer therapy. *Nat Rev Drug Discov* 2008 ; 7 : 231-40.
10. Roda JM, Joshi T, Butchar JP, *et al.* The activation of natural killer cell effector functions by cetuximab-coated, epidermal growth factor receptor positive tumor cells is enhanced by cytokines. *Clin Cancer Res* 2007 ; 13 : 6419-28.
11. Gowda A, Roda J, Hussain SRA, *et al.* IL-21 mediates apoptosis through up-regulation of the BH3 family member BIM and enhances both direct and antibody-dependent cellular cytotoxicity in primary chronic lymphocytic leukemia cells *in vitro*. *Blood* 2008 ; 111 : 4723-30.
12. Akamatsu N, Yamada Y, Hasegawa H, *et al.* High IL-21 receptor expression and apoptosis induction by IL-21 in follicular lymphoma. *Cancer Lett* 2007 ; 256 : 196-206.
13. Sarosiek K, Chen J, Pham DG, *et al.* Interleukin-21-induced apoptosis and cell death of diffuse large-B cell lymphoma (DLBCL) cell lines and primary tumors are associated with an induction of Bim. ASH Annual Meeting. *Blood* 2006 ; 108 (suppl) : 2503 (abstract).
14. Marzec M, Halasa K, Kasprzycka M, *et al.* Differential effects of interleukin-2 and interleukin-15 *versus* interleukin-21 on CD4+ cutaneous T-cell lymphoma cells. *Cancer Res* 2008 ; 68 : 1083-91.
15. Yoon J, Newton S, Wysocka M, *et al.* IL-21 enhances antitumor responses without stimulating proliferation of malignant T cells of patients with Sézary syndrome. *J Invest Dermatol* 2008 ; 128 : 473-80.
16. Iannello A, Tremblay C, Routy J, *et al.* Decreased levels of circulating IL-21 in HIV-infected AIDS patients : Correlation with CD4+ T cell counts. *Viral Immunol* 2008 ; 21 : 385-8.

NOUVELLE

# Migration des cellules tumorales : GEF et GAP montrent le chemin


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> Dans la plupart des cancers, la mortalité est essentiellement liée à la formation de métastases. Lors de ce processus, les cellules tumorales dont les fonctions prolifératives sont déjà altérées, complètent leur potentiel malin par un accroissement de leur capacité migratoire. Cela permet leur dissémination et l'établissement de tumeurs secondaires au niveau de sites distants. L'absence de stratégies thérapeutiques efficaces contre ce processus métastatique justifie l'effort de recherches et le besoin de découvertes dans ce domaine.

Dans l'organisme, les cellules sont en contact avec une matrice extracellulaire et évoluent dans un environnement en trois dimensions (3D). Les études récentes du processus de migration cellulaire à l'intérieur d'une matrice 3D (gels de collagène, matrices issues de cellules ou cultures associant plusieurs couches de cellules mimant la diversité d'un tissu) plus représentative de la réalité physiologique que les systèmes 2D classiques ont révolutionné la manière d'appréhender les mécanismes de motilité. Dans de tels environnements, la façon dont les cellules migrent et les morphologies qu'elles peuvent adopter sont complètement différentes de celles qu'elles adoptent lorsqu'elles sont cultivées sur les substrats bidimensionnels plats et rigides qui sont habituellement utilisés. Ainsi, en 3D, on distingue deux types de migration individuelle : la migration amiboïde et la migration mésenchymateuse [1]. Ces deux phénotypes migratoires ont également été observés *in vivo* et plusieurs études ont révélé que les cellules pouvaient alterner ces deux modes de migration, en fonction notamment des conditions environnementales [1, 2]. Si les conditions environnementales ne sont pas propices à l'un de ces modes de migration, certaines cellules cancéreuses opteront pour l'autre mode de migration afin de ne pas interrompre leur déplacement. Lorsque les cellules se déplacent selon le mode amiboïde, leur morphologie générale est celle d'une sphère dotée de protrusions membranaires globulaires extrêmement dynamiques. Dans ces conditions, les cellules semblent pouvoir migrer au travers de la MEC en s'affranchissant au moins partiellement de la dégradation protéique qui accompagne ce processus. *A contrario*, si les cellules choisissent une migration de type mésenchymateux, leur morphologie est allongée de type fibroblastique. Dans ce cas, la dégradation de la MEC est un prérequis pour que les cellules puissent la traverser.

### Rho et Rac déterminent le mode amiboïde ou mésenchymateux de la migration

Or, morphologie et migration sont sous la dépendance du cytosquelette d'actine et de l'ensemble des molécules capables de le remanier, notamment les petites GTPases de la famille Rho [8, 9]. Ainsi, les deux modes de migration dans une structure 3D sont déterminés par



Nouvelles.indd 19 25/03/2009 10:53:42

Le traitement de souris contenant des mélanomes par des cellules T $CD8^+$ spécifiques (transfert adoptifs), suivi d'une injection d'IL-21 conduit à un régression partielle de la tumeur lorsque l'IL-21 est utilisée seule [149]. Une régréssion complète est observée lorsque l'IL-21 est utilisée en combinaison avec d'autres cytokines (IL-2 et IL-15) [207]. Une récente étude menée par Pouw et al. démontre que la combinaison de l'IL-21 et de l'IL-15 sur des lymphocytes T murins exprimant un TCR dirigé contre la gp100 humaine (un antigène de mélanome HLA-A2 restreint) augmente la cytotoxicité des cellules T $CD8^+$, en augmentant l'expression de granzyme A et B et de la perforine [208].

De plus, ces deux cytokines combinées vont augmenter l'expression d'IL-2 et d'IFN-γ au niveau du sous-types de cellules T $CD8^+$ efectrices mémoires.

Une étude démontre aussi que l'IL-7 et l'IL-21 sont supérieures à l'IL-2 et l'IL-15 en ce qui concerne l'augmentation de la réponse anti-tumorale médiée par les cellules T dans un modèle de lymphome systémique dans des souris immunodéficientes [209].

Une étude récente a été réalisée dans un modèle de mélanome utilisant des cellules B16F10 transfectées par une IL-21 fusionnée à une ancre GPI (*Glycosylphosphatidylinositol*) et au GMCSF (*Granulocyte-Macrophage Colony Stimulating Factor*) [210]. L'IL-21-GPI-GMCSF a augmenté la cytotoxicité des cellules NK et des lymphocytes T cytotoxiques (LTC). De plus, cette IL-21-GPI-GMCSF a augmenté les niveaux sériques d'IFN-γ, a permis de contrôler la tumeur et a prolongé la survie des souris portant cette tumeur. Ces données proposent donc l'utilisation de cette IL-21 fusionnée dans le traitement des mélanomes [210].

Une étude réalisée sur de l'IL-21 fusionnée au GMCSF démontre que cette cytokine induit l'apoptose des lymphocytes T et B primaires exprimant l'IL-21R et ce d'une manière dépendante à l'activation de la protéine STAT-1 [211]. Les cellules B16 de mélanome exprimant cette cytokine ont été rejetées lorsqu'on les a implanté dans des souris C57BL6. De même, l'effet pro-apoptotique de cette cytokine a permis la survie des souris C57BL6 portant des lymphomes de type EL-4 [211].

Une étude menée par Kim-Schulze et al. démontre que l'IL-21 augmente les fonctions effectrices et l'activité anti-tumorale des cellules T $CD8^+$ endogènes ainsi que celles reçues lors de transferts adoptifs, et réduit l'activité des cellules T $CD4^+$ régulatrices au

niveau du micro-environement tumoral [212]. Bien que l'IL-21 utilisée seule possède un potentiel thérapeutique non négligeable, plusieurs groupes de recherche ont essayé de la combiner avec d'autres agents thérapeutiques [213]. Lorsque les souris porteuses de tumeurs ont été traitées avec l'IL-21 et un anticorps agoniste anti-TRAIL/DR5, une meilleure suppression de métastases ainsi qu'une augmentation d'une réponse médiée par les cellules T $CD8^+$ mémoires a été observée lors du second challenge tumoral [214]. De même, la combinaison de l'IL-21 avec de l'$\alpha$-GalCer (qui active les cellules NKT en se liant à leur récepteur CD1d) permet de réduire le développement de métastases [215]. Le transfert de cellules dendritiques pulsées avec de l'$\alpha$-GalCer, suivi par l'injection d'IL-21, ont permis de réduire des tumeurs métastatiques déjà établies [216]. D'une manière générale l'IL-21 peut donc augmenter les activités anti-tumorales des cellules NK, NKT, des cellules T $CD8^+$, et peut ainsi être utilisée en combinaison avec un grand nombre d'agents thérapeutiques différents. Ces études pré-cliniques ont donc démontré que l'IL-21 pouvait être utilisée contre différents types de tumeurs en impliquant différents types de réponses anti-tumorales. Ces études ont confirmé son potentiel anti-tumoral et ont conduit à son utilisation lors de plusieurs essais cliniques dans des stages avancés de mélanomes, de carcinomes à cellules rénales ainsi que lors de cancers du côlon et des ovaires [217, 218]. Selon leurs essais cliniques, l'IL-21 est bien tolérée par le corps humain. Son utilisation entraîne peu ou de faibles effets secondaires, contrairement à l'IL-2 et l'IFN-$\alpha$ qui engendrent des syndrômes capillaires ou une neurotoxicité. Ces études cliniques ont montré que l'IL-21 entraîne une augmentation d'expression de perforine et de granzyme B chez ces patients.

L'IL-21 peut restaurer le mécanisme d'ADCC au niveau des cellules NK de patients atteints de carcinome de cellules squameuses de l'œsophage [219]. Cette cytokine a pu en effet augmenter et finalement restaurer l'ADCC médiée par les deux anticorps utilisés dans cette étude qui sont le Trastuzumab (anti-HER2) et le Cetuximab (anti-récepteur à l'EGF) au niveau des cellules NK de ces patients.

Une étude originale et intéréssante a démontré l'effet de l'IL-21 sur l'angiogénèse des tumeurs [220]. L'IL-21 semble réduire la prolifération et la croissance des cellules endothéliales vasculaires activées. Lors d'études *in vivo* utilisant des membranes allantoïques d'embryons de poulets ainsi que des modèles murins de tumeurs, cette

équipe a démontré que l'IL-21 perturbe l'architecture des vaisseaux et affecte négativement la croissance de ces derniers. De plus, l'IL-21 réduit l'expression de plusieurs gènes reliés à l'angiogénèse [220].

Un autre effet anti-tumoral de l'IL-21 est celui médié directement par cette cytokine sur les cellules B. Une étude récente démontre aussi que cette cytokine arrête le cycle cellulaire et induit l'apoptose des cellules B larges et diffuses des lymphomes en activant les caspases [221]. De plus, l'IL-21 réduit la croissance de ces tumeurs lors de xénogreffes et prolonge la survie des souris greffées. Il semble que cet effet soit réalisé en régulant l'expression du facteur c-Myc. L'augmentation de ce facteur après traitement avec l'IL-21 réduit l'expression des protéines anti-apoptotiques Bcl-2 et Bcl-$X_L$.

Une étude similaire a démontré que l'IL-21 induisait l'apoptose des cellules B de lymphôme de manteau (*Mantle cell lymphoma*). La stimulation de ces cellules avec l'IL-21 a augmenté l'expression de trois protéines pro-apoptotiques (BIK (*Bcl2-Interacting Killer*), NIP3 (*BCL2/adenovirus E1B 19-kDa protein-interacting protein 3*), et HARAKIRI) et a réduit l'expression des protéines anti-apoptotiques Bcl-2 et Bcl-$X_L$ [222].

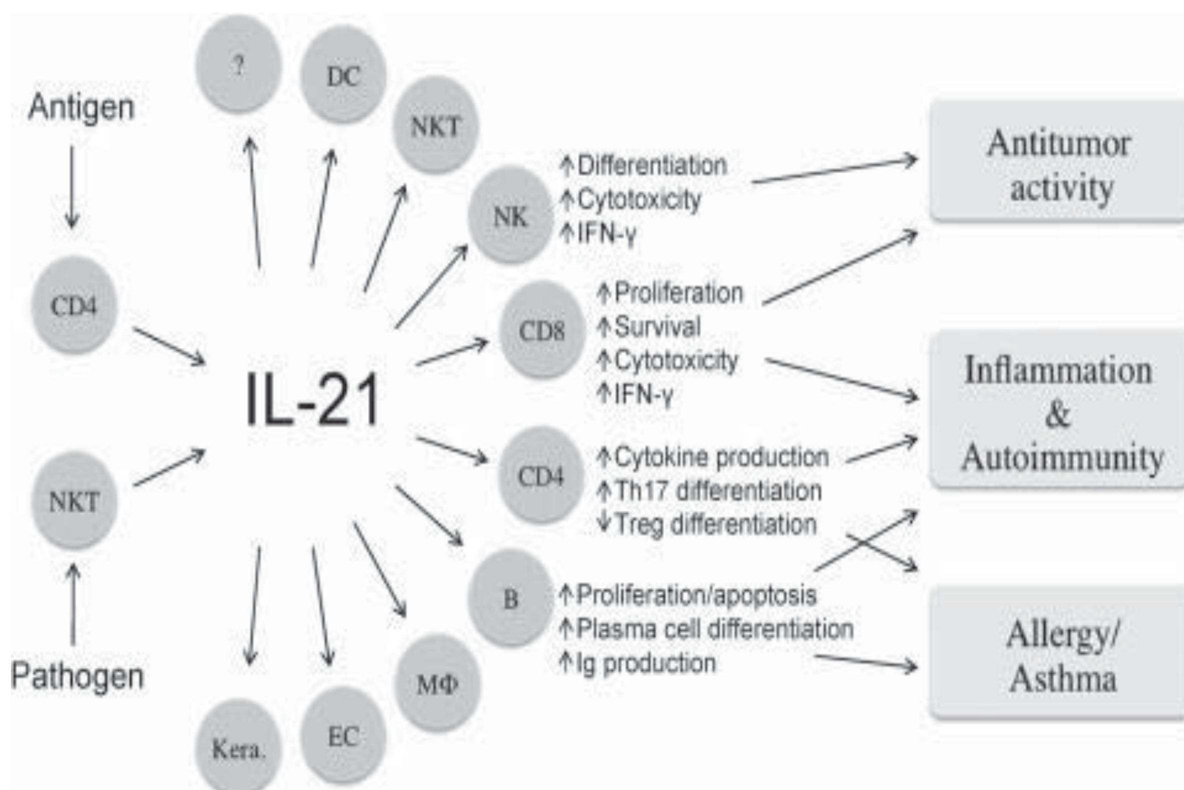
Cependant, une étude menée par Sondergaard et al. démontre que l'IL-21 endogène restreint de façon significative l'expansion des cellules $TCD8^+$ spécifique aux antigènes tumoraux et inhibe l'immunité médiée contre les lymphômes de type EG-7 exprimant l'ovalbumine ainsi qu'une expansion secondaire des cellules T $CD8^+$ mémoires [223]. Cette étude démontre d'une manière générale que le système endogène IL-21/IL-21R n'est pas requis pour l'immunité anti-tumorale médiée par les cellules NK, NKT et les cellules T $CD8^+$ et que l'IL-21 restreint même l'expansion des cellules $TCD8^+$ spécifique aux antigènes tumoraux et le rejet des tumeurs. Ceci propose donc un rôle immunosuppresseur pour cette cytokine.

L'effet de l'IL-21 sur le développement de la maladie du greffon contre le greffé (GvHD) a aussi été étudié. Les études démontrent que l'IL-21 joue un rôle important dans le développement du GvHD. En effet, une réduction du GvHD est observée lors de la transplantation de moelle osseuse contenant des splénocytes de souris IL-21R-/- par rapport aux splénocytes de souris sauvages [224, 225]. De même, l'injection de cellules

de moelle osseuse transduites avec une molécule servant de *decoy* récepteur pour l'IL-21 réduit l'apparition du GvHD [225]. L'étude menée par Bucher et al. démontre de façon similaire l'effet de l'IL-21 sur le développement du GvHD en expliquant que cette cytokine supprime la conversion des cellules T en cellules Trég FoxP3$^{+}$ [224].
Une étude portant sur les cellules T Vgamma9 Vdelta2a, le sous-type majeur de cellules $\gamma/\delta$ T réagissant contre les cellules tumorales en reconnaissant des phospho-antigènes, a démontré que l'IL-21 augmentait leur prolifération de façon limitée [226]. En combinaison avec l'IL-2, l'IL-21 a augmenté l'activité cytotoxique anti-tumorale de ces cellules, leur dégranulation, et a augmenté l'expression de CD56 et de plusieurs molécules ayant un rôle dans la lyse des cellules cibles. De plus, ces cellules exprimaient des niveaux réduits d'ILT-2 et de NKG2A qui sont des récepteurs inhibiteurs et exprimaient des niveaux faibles du récepteur NKG2D [226].

### 2-7 Rôle de l'IL-21 dans le contrôle des infections virales chroniques

L'induction d'une réponse immunitaire médiée par les cellules T CD4$^{+}$ et CD8$^{+}$ en conjonction avec les autres composantes du système immunitaire sont nécessaires pour contrôler les infections virales choniques. Il est aujourd'hui admis qu'en absence de l'aide apportée par les cellules T CD4$^{+}$, la réponse réalisée par les cellules T CD8$^{+}$ est compromise [227-229]. Malgré l'induction de la réponse médiée par les cellules T CD8$^{+}$ lors de la phase initiale d'une infection virale chronique, l'absence d'une réponse apportée par les cellules T CD4$^{+}$ est associée avec une incapacité des cellules T CD8$^{+}$ à développer leurs fonctions effectrices, ce qui résulte sur une absence dans le contrôle de l'infection [227-229]. Cependant, les éléments régulateurs clefs produits par les cellules T CD4$^{+}$ permettant d'obtenir une réponse antivirale complète et efficace étaient jusqu'à récemment mal compris voire inconnus.
Des études réalisées sur des modèles *in vivo* murins d'infection virale persistente (modèle basé sur l'infection de souris par le *Lymphocytic Choriomeningitis Virus* (LCMV)) ont démontré le rôle clef de l'IL-21 dans la réponse antivirale [230-232].




***(Leonard et al. J Leuk Biol. 2008 )***

**Figure 12. Conséquences et implications des effets biologiques de l'IL-21 sur les différentes composantes du système immunitaire**

Selon ces études, les effects biologiques de l'IL-21 sont nécessaires pour empêcher la baisse d'efficacité des cellules T qui jouent un rôle important dans le contrôle de l'infection. Lors de trois études majeures, les auteurs ont utilisé lors de leurs expériences deux souches différentes de LCMV (LCMV *Armstrong/Clone 13* ou LCMV *Aggressive/Docile*), qui possèdent un effet pathogène différent sur l'animal inoculé. L'injection de hautes doses de *Clone 13* ou de *Docile* vont résulter sur une résolution longue et difficile de l'infection alors que les infections par les LCMV *Armstrong* et *Aggressive* vont être résoluent rapidement lors de la phase aigüe de l'infection.

Lors de ces différentes études, les auteurs ont démontré que l'IL-21 était produite de façon prédominante dans les phases précoces et tardives de l'infection par les cellules T $CD4^+$ spécifiques au LCMV. Ils ont aussi montré que l'IL-21 maintenait les activités effectrices des cellules T $CD8^+$ spécifiques au LCMV requises pour le contrôle de l'infection. En effet, l'infection de souris KO pour l'IL-21R avec la forme immunosuppressive du LCMV a résulté sur une délétion prononcée des cellules T $CD8^+$ spécifiques au virus. Au contraire, ces souris n'ont eu aucune difficulté à résoudre l'infection lors de l'inoculation des souches *Armstrong* ou *Agressive*. Les auteurs ont démontré que les cellules T spécifiques au LCMV proliféraient normalement, conduisant ainsi à la génération de *pool* de cellules T spécifiques au LCMV qui ont permis une résolution de l'infection.

Pendant leurs études, ces groupes de recherche ont aussi démontré un effet direct de l'IL-21 sur la réponse antivirale médiée par les cellules T $CD8^+$ lors de l'infection chronique en utilisant deux différentes méthodes. Lors de l'utilisation de moelles osseuses chimères comprenant des moelles osseuses de souris sauvage ou KO pour l'IL-21R, ils ont démontré que les cellules T $CD8^+$ spécifiques au LCMV surpassaient en fonction et efficacité ces mêmes cellules provenant de moelles osseuses KO pour l'IL-21R. Deuxièmement, leurs études ont démontré que l'injection directe d'IL-21 dans des souris ne possèdant par de cellules T $CD4^+$ pouvait restaurer les fonctions antivirales médiées par les cellules T $CD8^+$ ainsi que réduire la charge virale chez les souris. Néanmoins, le traitement avec l'IL-21 était aussi associé avec 70% de mortalité chez les souris, suggérant un effet toxique potentiel de cette cytokine.

Leurs études ont permis de découvrir l'impact de l'absence de l'IL-21 sur la réponse hunorale après infection par le LCMV. En effet, suite à l'infection par les souches immunosuppressives du LCMV, une réduction significative dans la production d'anticorps spécifiques au LCMV a été observée chez les souris KO pour l'IL-21R. Ces observations sont consistantes avec les rôles clés médiés par l'IL-21 dans la génération des cellules plasmatiques et la production d'anticorps par ces dernières. De plus, ces données soulignent l'impact général et collectif de l'IL-21 dans la création et l'établissement d'une réponse immunitaire acquise et humorale lors d'une infection virale chronique. Néanmoins, des études additionnelles sont nécessaires afin de comprendre le rôle exact de l'IL-21 et l'interaction entre les cellules B et les cellules T $CD8^+$ lors de ces infections.

Le disfonctionnement des cellules T $CD8^+$ lors d'infections virales choniques a été fortement associé avec une expression soutenue du récepteur *Programmed Death-1* (PD-1) [233, 234]. Fröhlich et al. ont montré que PD-1 était fortement exprimé sur la majorité des cellules T $CD8^+$ spécifiques au LCMV chez les souris sauvages et KO pour l'IL-21R après infection [231].

Les auteurs ont aussi démontré que les niveaux sériques d'IL-10, une cytokine inhibitrice reconnue pour interférer avec la réponse médiée par les cellules T, ainsi que la fréquence de cellules productrices d'IL-10, augmentaient brusquement pendant la phase aigüe de l'infection chez les souris sauvages. La fréquence de cellules productrices d'IL-10, qui étaient principalement des macrophages, était basse chez les souris KO pour l'IL-21R, excluant donc un rôle pour cette cytokine dans l'épuisement des cellules T $CD8^+$ spécifiques au LCMV après infection [231].

Des etudes antérieures à celles-ci avaient démontré que l'infection virale persistente par ce virus résultait sur une augmentation de la production d'IL-10 par les cellules présentatrices d'antigènes, ce qui induisait au final sur une réponse altérée des cellules T [235, 236]. L'infection de souris ne produisant pas d'IL-10 (KO pour l'IL-10) résulte sur une réponse effectrice robuste médiée par les cellules T, une élimination rapide du virus, et le développement de cellules T antivirales mémoires. Des résultats similaires ont été obtenus lors de l'injection d'anticorps bloquant les effets biologiques de l'IL-10 suite à l'infection [236, 237]. L'IL-10 produite lors de l'infection inhibe les fonctions mémoires

et effectrices des cellules T $CD4^+$ dans le cas de l'infection aigüe qui est rapidement résolue [238]. Le fait d'inhiber cette cytokine permet donc de restaurer leurs capacités effectrices. Ces études suggèrent que l'IL-10 agit comme une molécule immunosuppressive conduisant à une persistence du virus, à un affaiblissement et un épuisement de la réponse antivirale médiée par les cellules T.

Ces études réalisées sur ces deux cytokines révèlent donc la complexité qui existe dans la compréhension du rôle des facteurs solubles nécessaires au développement d'une réponse antivirale rapide et efficace.

Le fait que l'IL-21 possède un rôle pléïotropique sur les différents bras de la réponse immunitaire nous amène à proposer cette cytokine comme le ou l'un des facteurs clefs fournit par les cellules T $CD4^+$ pour orchestrer la réponse immunitaire antivirale et promouvoir les fonctions effectrices des cellules T $CD8^+$ lors d'infections virales chroniques.

## 3- L'interleukine-18 (IL-18)

Les informations détaillées concernant cette partie ont fait l'objet de deux articles de revue publiés. Ces articles traitent de la biologie de l'IL-18, de son récepteur, de sa régulation lors de l'infection par le VIH-1, de son rôle dans l'immunopathogénèse du SIDA et de l'impact de son activité biologique au delà su système immunitaire. Ces articles ont été soumis à un processus de révision par les pairs avant leur publication.

**Article 2** : **Iannello A**, Samarani S, Debbeche O, Boulassel MR, Tremblay C, Toma C, Routy JP, and Ahmad A. Potential role of IL-18 in the immunopathogenesis of AIDS, HIV-associated lipodystrophy and related clinical conditions. 2009. ***Current HIV Research*** **8(2): 147-64**.

**Article 3** : **Iannello A**, Samarani S, Debbeche O, Boulassel MR, Tremblay C, Toma E, Routy JP, and Ahmad A. Role of Interleukin-18 in the development and pathogenesis of AIDS. 2009. ***AIDS Reviews*** **11(3): 115-25**.

**Résumé** : L'interleukine-18 est une cytokine pléïotropique pro-inflammatoire qui est souvent produite en réponse aux infections virales. Elle joue un rôle important dans la réponse immunitaire innée et acquise. Dans la circulation sanguine, l'IL-18 est liée à son antagoniste naturel appelée *Interleukin-18 Binding Protein* (IL-18BP). Cet antagoniste est induit de façon rétroactive en réponse à une augmentation de la production d'IL-18. Dépendamment du contexte, l'IL-18 peut augmenter la réponse Th1, Th2 ou Th17. Plusieurs groupes de recherche ont démontré que les concentrations et l'activité biologique de cette cytokine étaient augmentées chez les patients infectés par le VIH-1. Dans ces articles, nous discutons comment l'activité biologique de l'IL-18 peut contribuer au développement et à l'immunopathogénèse du SIDA. Nous commentons son impact sur le syndrôme de lipodystrophie associé au personnes infectées par le VIH-1 ainsi que son rôle dans les problèmes métaboliques observables chez ces patients. Nous discutons finalement de l'élaboration possible de stratégies ciblant le système IL-18/IL-18BP afin de traiter les patients infectés par le VIH-1.

# Article 2

**Iannello A**, Samarani S, Debbeche O, Boulassel MR, Tremblay C, Toma C, Routy JP, and Ahmad A. Potential role of IL-18 in the immunopathogenesis of AIDS, HIV-associated lipodystrophy and related clinical conditions. 2009. ***Current HIV Research* 8(2): 147-64**.

# Potential Role of IL-18 in the Immunopathogenesis of AIDS, HIV-Associated Lipodystrophy and Related Clinical Conditions

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**Abstract:** IL-18 is a pleiotropic and multifunctional proinflammatory cytokine that is often produced in response to a viral infection. The biological activities of the cytokine are tightly controlled by its natural antagonist, IL-18 binding protein (IL-18BP), as well as by activation of caspase-1, which cleaves the precursor form of IL-18 into its biologically mature form. The cytokine plays an important role in both innate and adaptive antiviral immune responses. Depending upon the context, it can promote TH1, TH2 and TH17 responses. Increased serum concentrations of IL-18 and concomitantly decreased concentrations of its natural antagonist have been described in HIV-infected persons as compared to HIV-seronegative healthy subjects. We discuss in this review article how increased biological activities of IL-18 contribute towards immunopathogenesis of AIDS, HIV-associated lipodystrophy syndrome and related metabolic disturbances. While the advent of potent anti-HIV drugs has significantly enhanced life span of HIV-infected patients, it has also increased the number of these patients suffering from metabolic disorders. The cytokine may prove to be a useful target for therapeutic intervention in these patients.

**Keywords:** Adipose tissue, AIDS, cytokines, HIV-1, IL-18, IL-18BP, lipodystrophy.

## INTRODUCTION

Viral infections usually induce production of several cytokines from the host cells. They do so by stimulating one or more so-called pattern recognition receptors (PRRs), which recognize certain pathogen-associated molecular patterns (PAMPs). The PRRs like Toll-like receptors (TLRs) occur on the cell surface and/or intracellularly in endosomal membranes and monitor extracellular environment and endosomes for the presence of PAMPs (reviewed in [1, 2]). The PRRs like nucleotide-binding and oligomerization domain (NOD)-like receptors (NLR), and Dex/DH box and CARD containing the retinoic acid-induced gene (RIG)-1 like RNA helicases (RHL) occur mainly in the cell cytoplasm and act as intracellular sensors for the PAMPs that have gained access to the interior of the host cells (reviewed in [3, 4]). A wide variety of bacterial and viral proteins, lipoproteins, nucleic acids, cell wall constituents, endo- and exotoxins, etc may act as PAMPs for one or more of the PRRs. After binding with their cognate PAMPs, the PRRs activate signaling cascades that culminate in the induction of several proinflammatory cytokines and chemokines from the host cells. The virus-induced cytokines and chemokines activate and chemoattract neutrophils, macrophages and natural killer (NK) cells in an attempt to kill and eliminate the infected cells. At the same time, they activate dendritic cells (DC) and initiate inflammatory and pathogen-specific immune responses [5, 6]. If the host immune responses successfully eliminate the pathogen, the cytokine production falls back to their base levels. However, pathogens like Human Immunodeficiency Virus type 1 (HIV) have evolved many immune escape mechanisms. Consequently, the infection persists and the host continues to produce cytokines, which may cause chronic inflammation, tissue destruction and overactive but ineffective immune responses (reviewed in [7, 8]). An optimal production of these cytokines is essential not only for the innate host resistance to pathogens but also for the induction, amplification and maintenance of the pathogen-specific immunity. The type of the cytokines induced in the host also determines the qualitative nature of the pathogen-specific immune response. It may induce differentiation of naïve $CD4^+$ T cells into predominantly TH1, TH2, TH17 or Treg type effector/memory cells (Fig. (**1**); reviewed in [9]). It has been well documented that the responses mediated by TH1 type cells are more effective in protecting the host from intracellular pathogens like viruses, whereas those mediated by TH2 type cells promote allergic responses to expel extracellular parasites and allergens. TH17 cells provide additional protection against extracellular bacteria and are potent inducers of inflammation, tissue destruction and autoimmunity.

A predominance of TH2 type cytokines was observed in HIV-infected/AIDS patients long ago and was postulated to be responsible for the inability of the host to eliminate and control the viral infection. Recent studies at single cell level also are in conformity with these earlier observations [9, 10]. It is believed that many of the immune abnormalities that occur in HIV-infected patients result from a dysregulated production of several immunologically important cytokines. For example, several researchers have reported decreased

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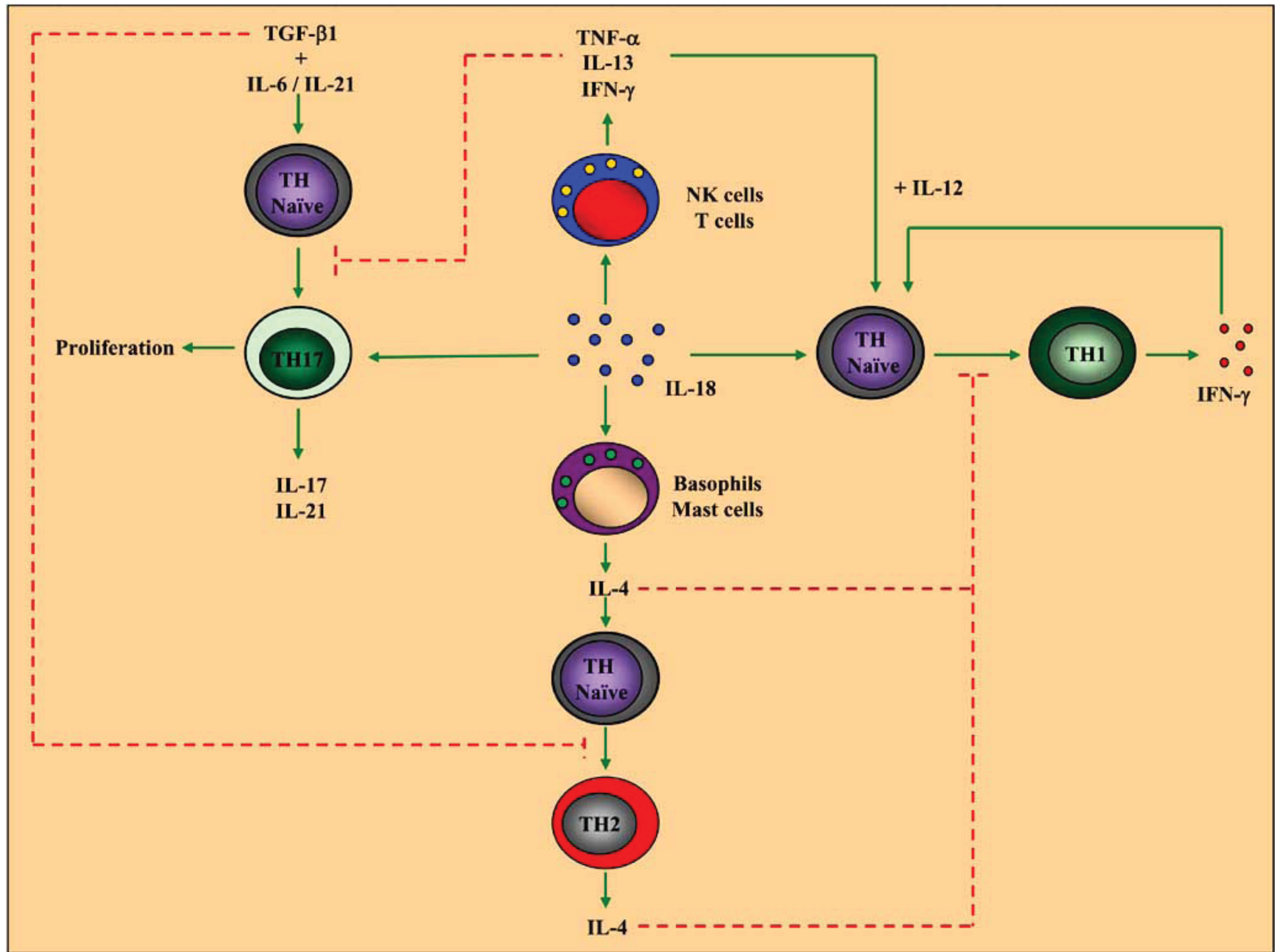


**Fig. (1). Effects of IL-18 on cytokine network regulating differentiation of naïve CD4$^+$ T cells.** Dashed lines denote inhibition or antagonism, and full ones indicate promotion of the development and differentiation process.

production of IL-12, IL-15 and IL-21, and increased production of TGF-β1 and IL-10 in HIV-infected/AIDS patients ([11-14]; reviewed in [15]). Several researchers including us have reported increased concentrations of IL-18 in the sera of HIV-infected/AIDS patients [16], (reviewed in [17]). This review discusses potential implications of the increased concentrations of this cytokine for anti-viral immune responses, pathogenesis and immunotherapy in HIV-infected AIDS patients. The review will begin with an overview of the IL-18 immunobiology.

## IL-18, ITS PRODUCTION AND SECRETION

IL-18 was first discovered in 1989 as the "IFN-γ-inducing factor" present in the serum of mice infected with the BCG strain of Mycobacterium bovis, and challenged with lipopolysaccharide (LPS). The factor induced IFN-γ production in IL-2-treated splenocytes taken from normal mice [18]. The cytokine was rediscovered, cloned and named as IL-18 in 1995 [19, 20]. It is a multifunctional and pleiotropic proinflammatory cytokine that is produced by activated macrophages, myeloid type dendritic cells, Kupffer cells, keratinocytes, articular chondrocytes, intestinal and airway epithelial cells, microglial and ependymal cells of brain as well as by certain neurons in the medial habenular nucleus. The gene is expressed in many tissues and organs including thymus, liver, lung, spleen, intestines, placenta, heart, kidney, brain, prostate, pancreas, adipose tissue, skeletal muscles, bone, cartilage, adrenal cortex and neurohypophysis (reviewed in [21, 22]). These cells and tissues usually express IL-18 gene and protein constitutively and increase their expression in response to infection, cold and LPS. Physical and mental stress can also stimulate IL-18 production *via* hypothalamus-pituitary-adrenal axis by releasing adrenocorticotropic hormone or ACTH [23]. Dendritic cells produce this cytokine constitutively and increase its secretion when they interact with other cells involving CD40/CD40L [24].

IL-18 belongs to the IL-1 family of cytokines (reviewed in [25]). All members of the family fold into a similar β-pleated trefoil structure. Like IL-1β and IL-33, IL-18 is produced as an inactive 24 kD precursor protein containing 192 amino acids. It has an unusual signal peptide of 35 amino acids and hence is not secreted *via* conventional secretory pathways. Within the cytoplasm, the precursor is cleaved on the carboxyl side of its aspartate 36 by the IL-1β converting enzyme (ICE) into mature biologically active 18 kD cytokine [26]. ICE-KO mice synthesize but do not process IL-18 precursor [27]. ICE is a member of a family of

cysteine-aspartate proteases called caspases, and is more commonly known as caspase-1. The caspase itself is also produced in the cells as an inactive 45 kD precursor, which must be activated before it could cleave the cytokine precursor. In addition to ICE; certain other proteases produced by mast cells (chymase) and neutrophils (proteinase 3, elastase, matrix metalloproteinase (MMP)-9, etc) can also cleave the extracellular IL-18 precursor into biologically active forms. Some proteases (e.g., caspase-3) may cleave the precursor into biologically inactive components. Thus, the secretion of biologically active mature IL-18 from macrophages requires two signals emanating from two distinct families of PRRs, (reviewed in [28, 29]; see Fig. **2**). The first signal comes from TLRs upon binding with their cognate ligands, and enhances transcription and translation of the precursor form. The second signal comes from a variety of stimuli and results in the assembly of certain NLRs into a multiunit complex called "inflammosome", which acts as a platform for activating caspase-1, processing of IL-18 precursor and its secretion from the cells (reviewed in [28, 29]). The requirement of the two distinct signals for the secretion of biologically active IL-18 is probably a safeguard mechanism to prevent excessive production of this cytokine that may cause tissue damage. A well-studied model of the second signal is binding of ATP to a purinergic receptor P2X7R, an ATP-gated ion channel, expressed on the surface of IL-18 producing cells, e.g., macrophages ([30]; see below). The second signal also causes secretion of the cytokine from the cells in exosomes as mutivesicular bodies [30].

A typical NLR (also known as Caterpiller, NOD, NOD-LRR or NACHT-LRR) is characterized by the presence of a C-terminal ligand-binding leucine-rich repeats (LRR), a centrally located nucleotide binding and oligomerization (NOD) domain, and an N-terminal effector (Pyrin, CARD: Caspase recruitment domain or BIR: Baculovirus inhibitor of apoptosis repeat) domain. To date more than twenty distinct members of the NLR family have been identified (reviewed in [3, 31]). Only a few members of the family assemble into inflammosomes, while others form multiprotein complexes called apoptosomes and nodosomes, which regulate cell death, and activate NF-κB, respectively. Still others may act as accessory proteins to scaffold the multiunit NLR complexes. Three NLR members, NALP-1, NALP-2 and NALP-3 assemble into inflammosomes and recruit precursor caspase-1 into these complexes *via* an adaptor and scaffolding protein ASC (Apoptosis-associated Speck-like protein containing a Caspase recruitment doomain) and related proteins, which help in the oligomerization and protein-protein interactions within the complexes. Normally NLRs exist in auto-inhibited monomeric forms. A variety of stimuli cause their assembly into inflammosome accompanied by activation and auto-cleavage of caspase-1 into its mature form, which then cleaves the precursor forms of IL-18 and related cytokines (IL-1β and IL-33). It is not yet clear how NALP proteins recognize their ligands *via* their LRR domains and how these ligands get access to these receptors within the cytoplasm of the cells. Recent studies have shown that a diverse array of substanses, e.g., phagocytosed urate and silica crystals, aluminum compounds, amyloid β crystals, intracellular bacteria and their products, bacterial and viral nucleic acids, etc can cause activation of caspase-1 and secretion of active IL-1β [32-36]. Some pore-forming bacterial toxins, e.g., listeriolysin O, nigericin and maitotoxin can also cause activation of caspase-1. It is believed that these toxins open channels within the plasma membrane of the cell giving access to various ligands to NLRs and/or cause efflux of $K^+$ from the cells. The binding of extracellular ATP to P2X7R causes translocation of a protein pannexin-1 (a hemichannel) from the cytoplasm to the receptor. The channel provides access to extracellular ATP to the cell interior. It also allows efflux of $K^+$ from the cell. NLRs are believed to sense this efflux. It appears that some infammosomes may sense cell integrity *via* rupture of lysosomal membranes, as the rupture may allow lysosomal constituents like cathepsin B to gain access to the cell interior. Direct intracellular injection of cathepsin B and rupture of lysosomal membranes have also been shown to cause inflammosome assembly and activation of caspase-1 [29, 32]. Interestingly, the cells undergoing necrosis, but not apoptosis, may also activate certain infammosomes and secrete IL-1β and IL-18 [37]. The recognition of a wide array of structurally diverse ligands *via* structurally similar LRR domains of various NALPs remains enigmatic. The assembly of certain "nodosomes" could also trigger oligomerization of certain "inflammosomes" and hence processing and secretion of mature IL-18 and related cytokines. For example, muramyl dipeptide (MDP), derived from the bacterial cell wall proteoglycan, binds NOD-2 in the host cell cytoplasm and activates caspase-1 *via* an inflammosome that contains NALP-1 [38].

The exact molecular mechanisms that couple the cleavage and secretion of the mature IL-18 (and related cytokines) are not completely understood. It has been shown that cellular oxidation *via* nicotinamide adenine dinucleotide phosphate (NADPH) oxidase plays an essential role in the ATP-mediated secretion of mature cytokines. Thus, the reactive oxygen and nitrogen species that increase NADPH oxidase activity also result in the processing and secretion of IL-18. Antioxidants, however, only partially block this oxidation. Since free ATP does not exist outside cells and only stressed and injured cells release it, IL-18-producing cells sense it as a "danger signal" and respond by secreting proinflammatory cytokines. It is noteworthy that along with the cleaved cytokine, some precursor cytokine is also secreted. This may be degraded or activated depending upon the type of the proteases present in the extracellular milieu. In the presence of inhibitors of caspase-1, ATP causes secretion of only precursor IL-18. Gain-of-function mutations in certain NALPs have been documented. As expected, the individuals with these mutations suffer from a variety of chronic autoinflammatory diseases (reviewed in [3, 22, 31]).

## IL-18 RECEPTOR

IL-18 exerts its biological effects on target cells *via* a specific receptor called IL-18 receptor (IL-18R), which is expressed on a wide variety of body cells, e.g., vascular endothelial cells, NK, NKT, B, dendritic and TH1 type $CD4^+$ T cells, basophils, mast cells, synovial fibroblasts, adipocytes, etc [39, 40]. The receptor comprises two units, α and β chains, whose extracelluar and intracellular regions contain Ig-like and Toll/IL-1R (TIR) domains, respectively. Extensive mutagenesis studies have shown that IL-18 has

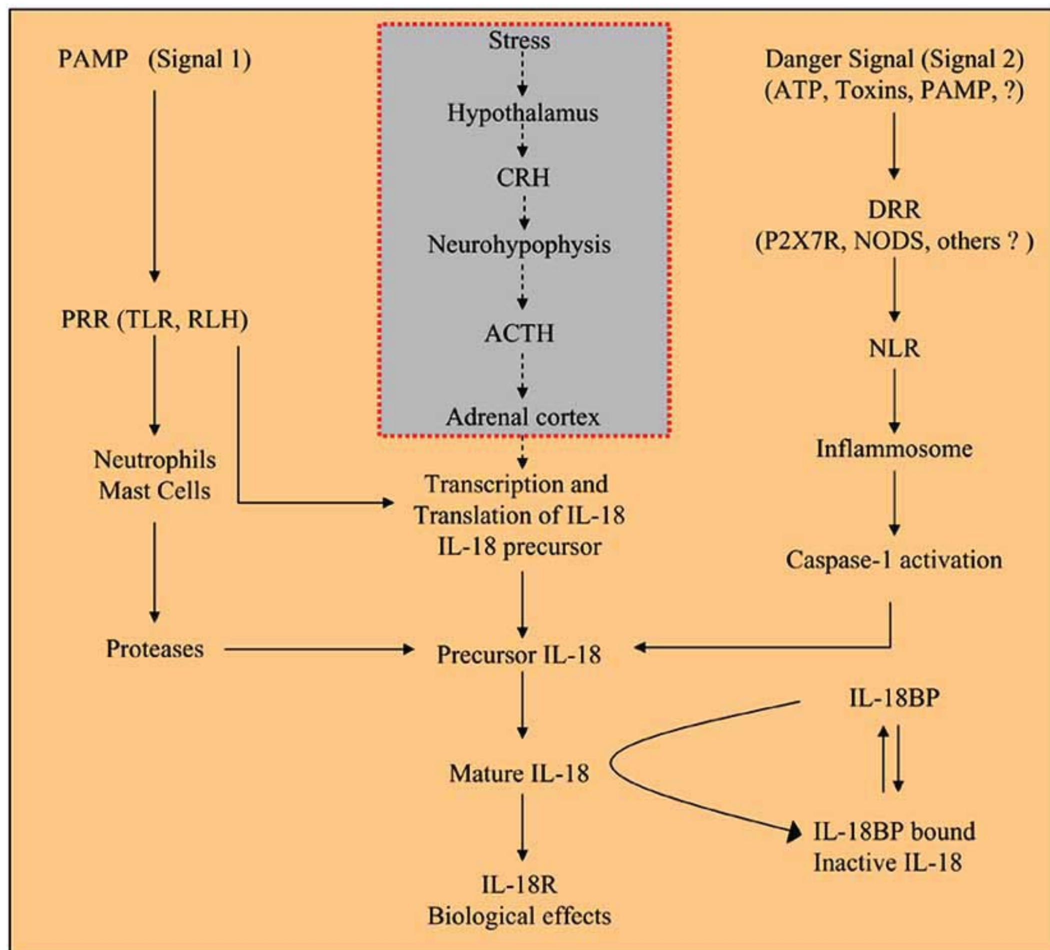


**Fig. (2). Signals needed for production of biologically active IL-18.** Two signals needed for the production of biologically active IL-18 from various human cells are depicted. The stress induces IL-18 production *via* endocrine (more specifically hypothalamus-pituitary-adrenal or HPA) pathway. ACTH: Adrenocorticotropic hormone, CRH: Corticotropic releasing hormone, DAMPS: Danger-associated molecular patterns, DRR: Danger recognition receptors, PRR: Pattern recognition receptors, RLH: RIG-like RNA helicases. A question mark (?) indicates unknown signals or receptors.

three binding sites for its receptor: Two bind with the IL-18Rα chain and the third binds with the IL-18Rβ chain [41]. Upon binding of IL-18 to the receptor, the TIR domains transduce signals *via* MyD88-IRAK-TRAF-6, and activate NF-κB and MAPK (ERK1/2, p38 and JNK; reviewed in [22]). The cytokine can also activate PI3-K and other pathways in MyD88-independent fashion (see details in Fig. **3**).

## CONTROL OF IL-18 ACTIVITY IN THE BODY

IL-18 is a powerful proinflammatory cytokine. Its biological activity is tightly controlled *in vivo* by a naturally occurring antagonist, IL-18 binding protein (IL-18BP; [42, 43]). The antagonist-bound IL-18 can bind to the "α" chain of the IL-18R but cannot recruit its β chain and transduce signals. The antagonist is produced constitutively in the human body by a wide variety of cells and tissues. The protein is produced as a negative feed back mechanism in response to IL-18. In this regard, IL-18-induced IFN-γ acts as a powerful stimulus for the production of this protein. Gamma Interferon-activated sequences (GAS) and interferon regulatory factor-response elements (IRF-RE) are present in the promoter region of the IL-18BP gene [44, 45]. The human IL-18BP exists in four isoforms designated "a", "b", "c" and "d". The isoforms result from alternate splicing of the mRNA [42]. Of these isoforms, only "a" and "c" bind with, and inactivate IL-18. Furthermore, the "a" isoform binds IL-18 with 10-fold higher affinity than the "c" isoform, and accounts for most of the IL-18 neutralizing activity in the circulation of human body. IL-18BP bears no homology with IL-18R. It binds with IL-18 in 1: 1 stoichiometry with an affinity that is several orders of magnitude higher than that of IL-18 for its receptor. The high affinity complex formed has a very low dissociation rate. At equimolar concentrations, the "a" isoform can neutralize about 50% IL-18, reaching up to 95% at a molar excess of 2. The circulating levels of IL-18 increase sharply in sepsis; however, most of it exists in inactive state, bound with IL-18BP [46]. In *in vitro* experiments, IL-18BP impairs TH1 responses by inhibiting IL-18-mediated production of IFN-γ and activation of NF-κB [43, 47]. By inhibiting IL-18-induced IFN-γ production, IL-18BP promotes prostaglandin production from human PBMC [48]. It protects mice from collagen-induced arthritis [49]. The antagonist may also protect the host from viral infections by neutralizing the virus-induced IL-18. Therefore, it is not surprising that

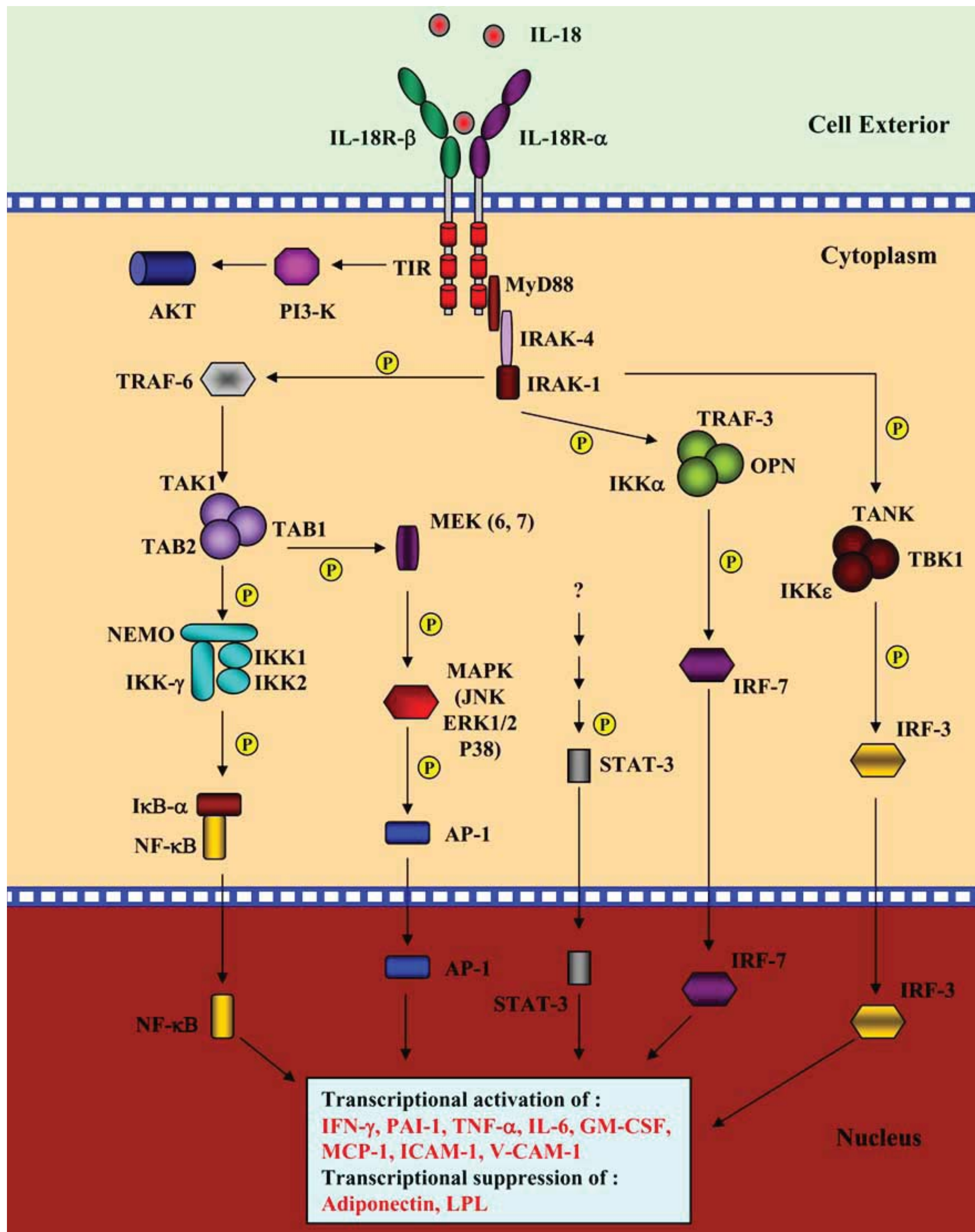


**Fig. (3). IL-18-induced signaling cascade.** Upon binding of mature IL-18 to its receptor, TIR domains present in the cytoplasmic domains of the receptor chains recruit IRAK-4 *via* adapter protein MyD88. IRAK-4 autophosphorylates, dissociates from the complex and activates IRAK-1, which in turn phosphorylates and activates E3 ligases, TRAF-6 and TRAF-3. IL-18 activates three major signaling pathways, NF-κB, MAPK and IRF as depicted in the Figure. The pathway leading to STAT-3 activation is unknown. The genes activated or repressed by the IL-18-induced signaling in various human cell types are also shown. IRAK: IL-1 receptor-associated kinase, TRAF: TNF receptor-associated factor, IKK: Inhibitor of IκB kinase, OPN: Osteoprotein precursor, TAK-1: TANK (TRAF-associated NF-κB activating kinase) activating kinase-1, TAB: TAK-1 binding protein, AP-1: Activation protein-1, IRF: Interferon regulatory factor, GM-CSF: Granulocyte-macrophage-colony stimulating factor, LPL: Lipoprotein lipase.

human poxviruses, ectromelia, vaccinia and cowpox have usurped the IL-18BP gene and have incorporated it into their genomes. The viral gene acts as a virulence factor, as its expression attenuates host's IL-18 response. The poxviruses with mutant viral IL-18BP gene become relatively avirulent in mice [50, 51]. The viral IL-18BP is immunosuppressive and has anti-inflammatory properties [50].

Another member of the IL-1 superfamily, IL-1F7, is also processed by casapse-1. Its splice variant, IL-1F7b, complexes with IL-18BP and enhances its IL-18-neutralizing activity. It can also bind the IL-18Rα chain weakly but cannot recruit the β chain and transduce signals ([52]; reviewed in [53]).

## BIOLOGICAL ACTIVITIES OF IL-18

As mentioned above, IL-18 is a member of the IL-1 family. All members of the family usually induce fever and exert pro-survival effects on target cells. However, IL-18 is a unique member of the family, as it does not induce fever and has proapoptotic effects [22, 54]. IL-18 exerts many other biological effects: it induces production of IFN-γ, TNF-α, IL-8, IL-1β, MIP-1α, NO and matrix metalloproteases from several cell types including T and NK cells; induces expression of adhesion molecules on vascular endothelial cells; promotes maturation of NK and T cells and increases their cytotoxicity *via* increased expression of FasL and perforin; regulates pathogen-specific immune responses; and plays a role in the homeostasis of energy (see Table **1**). The cytokine increases expression of perforin without affecting expression of perforin mRNA [55]. Neutralization of IL-18 prevents accumulation of neutrophils in the lungs and liver of mice in otherwise lethal endotoxaemia induced by LPS from Salmonella typhimurium and Escherichia coli [56, 57]. Professional antigen presenting dendritic cells (DC) produce IL-18 as well as express its receptors. IL-18 chemoattracts DC to the site of viral infection and induces their maturation directly and indirectly [58]. IL-18 plays an important role in intercellular communication among DC, NK, T, B and endothelial cells. It induces expression of OX40L (CD134L) on DC. The CD134L-expressing dendritic cells induce differentiation and expansion of IFN-γ producing antigen-specific T cells [59]. The cytokine is the most powerful stimulus for IL-12-induced production of IFN-γ from NK and T cells. A coordinate secretion of these two cytokines is very effective in invoking TH1 type immune responses. IL-18 and IL-12-induced IFN-γ causes expression of IL-12Rβ1 chain on naïve T cells and makes them responsive to IL-12 and differentiation into TH1 type $CD4^+$ effector/memory T cells. Recent studies have shown that IFN-γ also prevents differentiation of TH17 type $CD4^+$ T cells, which are the most powerful inducers of inflammatory conditions and tissue destruction in the host (reviewed in [60, 61]). This may explain protective effects of IL-18-induced interferon from certain models of autoimmune and chronic inflammatory diseases. The interferon also induces antiviral state in the host cells. It can also induce apoptosis of virus-infected cells by modulating expression of TRAIL and TRAIL receptors on human cells [62]. The protective effects of IL-18-induced IFN-γ have been demonstrated in mice from HSV-1 infection [63].

IL-18 can effectively promote TH1 type responses by inducing proliferation of TH1 type $CD4^+$ T cells (reviewed in [64]). However, the cytokine *per se* does not induce differentiation of TH1 cells. Naïve T cells only express the ligand-binding IL-18Rα chain. The ability of IL-18 to amplify TH1 type responses depends upon IL-12, which induces expression of IL-18Rβ chain on naïve T cells during their differentiation into TH1 type $CD4^+$ T cells [65]. Interestingly, in the absence of IL-12, IL-18 promotes TH2 type immune responses. The cytokine does so by inducing production of IL-4, IL-5, IL-9 and IL-13 from basophils and mast cells (reviewed in [66]). These cells constitutively express IL-18R and produce these cytokines in response to IL-18. It is noteworthy that IL-4 promotes differentiation and development of TH2 type $CD4^+$ T cells. IL-13 induces goblet cell hyperplasia and mucus secretion, whereas IL-5 causes eosinophilia [66]. IL-18 also induces secretion of IL-4, IL-9 and IL-13 from ligand-activated NKT cells [67, 68]. Even fully differentiated TH1 type $CD4^+$ T cells secrete TH2 type cytokines in addition to IFN-γ, when they undergo antigen-specific stimulation in the presence of IL-18 [69]. Under these conditions, B cells undergo differentiation and produce IgE [66]. Thus, IL-18 has the potential to induce TH2 type responses, which promote allergic immediate hypersensitivity type responses needed for expulsion of multicellular parasites. These effects of IL-18 cannot be inhibited even by the presence of IL-12. This explains why viral infections are generally accompanied by both TH1 and TH2 type responses and exacerbate allergic diseases like

**Table 1. Biological effects of IL-18**

| |
|---|
| 1. It induces, together with IL-12, production of IFN-γ from NK and T cells [65, 169-171]. |
| 2. It plays a role in the interactions of DC with other cell types [58, 172, 173]. |
| 3. With IL-12, IL-18 suppresses, and without it, promotes IgE synthesis [174, 175]. |
| 4. It induces proliferation of TH1 and TH17 type $CD4^+$ effector/memory cells [61]. |
| 5. It activates and induces production of IL-4, IL-13 and histamine from mast cells and basophils [66, 176]. |
| 6. IL-18 converts TH1 type $CD4^+$ T cells into TH2 type cytokine-producing cells upon antigenic stimulation [69]. |
| 7. It induces FasL expression on NK cells [91, 177]. |
| 8. It enhances perforin expression in NK and T cells without affecting transcription [55, 178]. |
| 9. It induces TNF-α, IL-6, IL-1β, NO, MCP-1 and PAI-1 production from a variety of human cells [54, 64]. |
| 10. It enhances adhesion and extravasation of blood cells by inducing expression of ICAM-1 and VCAM-1 [137]. |
| 11. IL-18 causes accumulation of neutrophils at inflammatory sites [56, 57]. |
| 12. It affects energy homeostasis and lipid metabolism [114, 166, 179]. |
| 13. It stimulates production of matrix metalloproteinases and tumor metastasis [180]. |
| 14. It induces IL-4 from ligand-activated NKT cells [67, 68]. |
| 15. It induces HIF and VEGF, and modulates angiogenesis [181, 182]. |
| 16. It promotes cartilage degradation but inhibits bone erosion [180, 183-185]. |

DC: Dendritic cells; HIF: Hypoxia-induced factor; NO: Nitric oxide; PAI-1: Type 1 plasminogen activator inhibitor; VEGF: Vascular endothelial growth factor.

asthma. The ability of IL-18 to promote both TH1 and TH2 type cytokine responses in a context-dependent manner has earned it the name of a "switch" cytokine. The usage of this name should be discouraged, as the cytokine does not promote one or the other type of responses. Rather, it promotes both of them in parallel.

Despite a protective role of IL-18 against viral, bacterial and fungal infections, chronic and excessive production of the cytokine may cause tissue destruction and contribute towards virus-induced immunopathology. It is important to note that increased levels of IL-18 have been reported in the circulation of patients suffering from chronic inflammatory and autoimmune diseases like multiple sclerosis, hepatitis, Crohn's disease, graft versus host disease, psoriasis, rheumatoid arthritis, etc. The cytokine plays an important role in the etiopathogenesis of these diseases (reviewed in [54]).

As the cytokine is produced in brain, pituitary and adrenal glands in response to stress and cold, it mediates communication among CNS, endocrine and immune system. The cells in the adrenal cortex that produce glucocortoids also produce IL-18. Furthermore, being produced in adipose tissue, IL-18 could play a role in energy and lipid homeostasis (see below).

## IL-18 PRODUCTION IN HIV INFECTION

As mentioned above, IL-18 is an integral part of the cytokine profile that is produced in response to a viral infection. Many viruses and intracellular pathogens induce its production from host cells and consequently increase IFN-γ production from NK and T cells (reviewed in [70, 71]). This early induction of IFN-γ is important for inducing a TH1 type antiviral response and in inhibiting TH17 type strong inflammatory responses. The induction of IL-18, in response to a viral infection, is at least in part responsible for virus-induced activation of NK cells in the host. It appears that like many other viruses, HIV-1 also induces IL-18 response in the host. A close cousin of HIV-1, SIV, induces the expression of this and other cytokine genes in experimentally infected Rhesus monkeys [72]. We could not find any study in literature that has investigated a direct effect of the virus on IL-18 production from human PBMC, dendritic cells or macrophages. However several researchers have documented increased concentrations of this cytokine in the circulation of HIV-infected persons. Torre *et al.* first described increased concentrations of IL-18 in the sera of HIV-infected symptomatic and AIDS patients but not in asymptomatic persons as compared with these levels in healthy subjects. Furthermore they found no correlation of the cytokine levels with viral load, $CD4^+$ T cell counts or opportunistic infections in AIDS patients [73]. These results have been verified and extended by several researchers including us ([16, 74]; reviewed in [17]). We showed that the patients' sera contained on the average three-fold higher concentrations of this cytokine as compared to their control counterparts. The increased IL-18 concentrations were associated with their increased biological activities when they were tested for their ability to induce IFN-γ from human T cells. We could not find any correlation of these elevated serum concentrations with $CD4^+$ T cell counts, $CD8^+$ T cell counts, the ratio between these two cell counts or with the plasma viral loads of these patients [16]. However, Stylianou *et al.* [74] found a significant correlation between concentrations of this cytokine and viral loads of the patients. They also found that anti-retroviral therapy decreased viral load as well as the levels of this cytokine; however, they always remained above normal values. The workers suggested that increased concentrations of the cytokine despite anti-retroviral therapy (ART) might indicate treatment failure. Collectively, these results suggest that HIV, its gene products and/or nucleic acids may induce enhanced production of this cytokine in HIV-infected individuals. In keeping with this notion, Sailer *et al.* reported higher levels of the cytokine in HIV-infected persons early in the course of the infection [75].

## SOURCE OF IL-18 IN HIV-INFECTED PERSONS

Despite increased levels of IL-18 in the circulation of HIV-infected persons, their PBMC were reported to produce less amounts of this cytokine constitutively as well as in response to LPS and Staphylococcal bacteria [16, 76, 77]. The decreased production occurred both at the protein and mRNA levels. David *et al.* noted correlation between the decreased IL-18 and IL-2 mRNAs in the PBMC of HIV-infected patients [76]. Interestingly, the production of IL-18 from the PBMC in response to LPS correlated with the serum levels of the cytokine in HIV-seronegative healthy subjects but not in HIV-infected individuals. The expression of caspase-1, which processes IL-18 precursor into its biologically active form, was also reduced in the PBMC of HIV-infected persons as compared to those in HIV-seronegative healthy control subjects [16]. The reduction was seen both in the precursor and activated forms of the protease, and was not due to reduced numbers of monocyte-macrophages in HIV-infected persons. In this connection, we, and others have shown that a decreased production of IL-18 from the PBMC of HIV-infected persons was due to high concentrations of TGF-β1 in the circulation of these patients [16, 77]. We also found a significant negative correlation between IL-18 production from the PBMC of HIV-infected persons and their serum TGF-β1 levels. Neutralizing this cytokine with cytokine-specific antibodies significantly augmented their ability to produce IL-18 constitutively as well as in response to LPS [16]. It was also shown that IL-10 could play a similar role in suppressing IL-18 and IL-1β production from the PBMC in these patients [77]. Interestingly, mononuclear cells from the placenta of HIV-infected women were also compromised in their ability to produce this cytokine [78]. It may be relevant to mention here that the concentrations of both IL-10 and TGF-β1 are increased in the circulation of HIV-infected persons [15].

Despite a decreased production of IL-18 from the PBMC of HIV-infected persons, it is not clear what contributes to the increased concentrations of this cytokine in their circulation. In an attempt to find a source of increased IL-18 in the circulation of HIV-infected persons, we discovered that human platelets contain abundant amounts of this cytokine and release it upon activation into the circulation [79]. Platelet activation occurs in HIV-infected persons and this activation contributes to increased IL-18 levels present in the circulation of these individuals. In this regard, we observed a significant positive correlation between platelet activation and circulating IL-18 levels in HIV-infected

persons [79]. It is noteworthy that IL-18 is also induced in response to anxiety, physical and psychological stress *via* hypothalamus-pituitary-adrenal (HPA) axis in animal models. Given that HIV-infected persons undergo enormous amounts of emotional and psychological stress, these organs could potentially contribute to increased IL-18 levels in the circulation. Furthermore, intestinal epithelial cells also contain preformed IL-18 and release them in response to a variety of stimuli [54, 80]. A wide spread infection of $CD4^+$ T cells and macrophages present in the gastrointestinal tract could also stimulate the release of this cytokine from the intestinal epithelial cells [81].

## IL-18BP IN HIV-INFECTION

As stated above most of the circulating IL-18 is bound with IL-18BP and is biologically inactive. This protects the body from potentially harmful proinflammatory effects of the cytokine. We found a severe imbalance in the production of IL-18 and IL-18BP in HIV-infected persons (Iannello *et al.* submitted; reviewed in [82]). The serum concentrations of these two soluble mediators correlate with each other in HIV-seronegative healthy subjects. However no such correlation existed between them in HIV-infected persons. Contrary to the concentrations of IL-18, those of IL-18BP were significantly decreased in HIV-infected persons as compared to their healthy counterparts. An increase in the serum concentrations of IL-18 was not accompanied by a corresponding increase in its antagonist. Rather the concentrations of the antagonist were decreased in these sera. Consequently, the concentrations of biologically active free IL-18 were further increased in the sera of HIV-infected persons. Indeed we have earlier reported increased biological activity of IL-18 in the sera of HIV-infected AIDS patients [16]. The caveat is that these results were based upon the measurement of only "a" isoform of IL-18BP. However, since this isoform is mainly responsible for neutralization of IL-18 in humans, it is reasonable to conclude that IL-18BP is decreased in these patients. As mentioned above, this protein is produced as a negative feed back mechanism in response to IL-18-induced IFN-γ. Decreased production of IL-12, IL-15 and IL-21 has been reported in HIV-infected persons (reviewed in [15]). In the absence/deficiency of these cytokines, IL-18 has little capacity to induce IFN-γ production. Indeed a decreased production of IFN-γ has been documented in the circulation of HIV-infected persons ([83, 84]; see below). The decreased production of this interferon may be at least in part responsible for decreased production of IL-18BP in these patients. Furthermore, we have obtained evidence that HIV-1 infection inhibits production of IL-18BP in human monocyte-derived macrophages (Iannello *et al.* submitted; reviewed in [82]). This may further explain the imbalance in the production of IL-18 and IL-18BP in HIV-infected patients. Imbalance in the production of IL-18 and its natural antagonist has also been reported in secondary haemophagocytic syndrome (SHS), hepatic cirrhosis, Crohn's disease, lupus nephritis and sepsis in humans [46, 85-88]. In these conditions, the levels of IL-18 increase in the circulation, but these increases are not accompanied by corresponding increases in the production of IL-18BP. Consequently, the levels of free biologically active cytokine are more increased than those indicated by measurements of the total cytokine. For example, Novick *et al.* [46] have reported that the levels of IL-18 are increased by 20-fold as compared with a 10-fold increase in levels of IL-18BP in sepsis patients. This imbalance in the production of these two soluble mediators results in increased levels of free biologically active IL-18.

## EFFECTS ON ANTIVIRAL IMMUNITY

As mentioned above, the ability of IL-18 to promote TH1 responses depends upon IL-12, which induces expression of IL-18R on TH1 type $CD4^+$ T cells. In the absence/deficiency of IL-12, IL-18 acts on mast cells, basophils, and NKT cells, and induces production of IL-4, which promotes TH2 type responses in the body (reviewed in [66]). A decreased production of IL-12 has been well documented in HIV-infected persons. The defective production occurs early in the course of the infection [13, 15]. In addition to IL-12, decreased production of IL-15 and IL-21 has been well documented in the infected persons [11, 14, 15]. These are the cytokines that work in synergy with IL-18 for inducing IFN-γ and other cytokines from NK and T cells. Therefore it is very unlikely that IL-18 could promote increased IFN-γ production and consequently decrease HIV replication in these patients. Decreased numbers of IFN-γ producing, but not of IL-4-producing, T cells as well as decreased levels of IFN-γ have been observed in the circulation of HIV-infected persons [83, 84] and support this notion. A decreased production of this interferon could also occur due to increased production of other immunosuppressive cytokines e.g., TGF-β1 and/or IL-10, in these patients (reviewed in [10, 15]). A decreased production of IFN-γ and a predominance of TH2-like responses in this infection have been incriminated in the pathogenesis of AIDS (reviewed in [89]).

Increased IL-18 concentrations have been reported in the circulation of several chronic inflammatory conditions and infections. These conditions are often accompanied by decreased numbers of NK cells as well as by their reduced cytotoxic activities [88, 90]. Interestingly, researchers have discovered an inverse correlation between serum IL-18 levels and NK cell numbers in these patients. It appears that IL-18 somehow resulted in the depletion of NK cells in these patients [88, 90]. We found a similar inverse correlation between serum IL-18 concentrations and NK cell numbers in HIV-infected persons [91]. This correlation existed between the cytokine and all major subsets of NK cells: $CD3^-CD56^+$, $CD3^-CD16^+$ and $CD56^+CD16^+$. Furthermore, we found that the cytokine induced fratricidal killing of NK cells by a mechanism that involved Fas-FasL interactions and TNF-α. Recombinant human IL-18 induced expression of FasL on the cell surface in primary human NK cells as well as in an established human NK cell line. The cytokine stimulates the FasL gene promoter and hence increases transcription from the gene. Furthermore, the cytokine increases sensitivity of human NK cells to apoptotic stimuli by decreasing expression of an anti-apoptotic protein Bcl-$X_L$, without affecting the expression of Bcl-2. Although IL-18-treated NK cells may increase killing of Fas-expressing target cells, they may also kill other NK cells, which constitutively express Fas on their surface. It is noteworthy that decreased NK cell numbers and their compromised functional activities have been well documented in HIV-literature (reviewed in [15]). Thus, increased IL-18 concentrations may play a role

in causing defects in the NK cell compartment in HIV-infected patients.

## EFFECTS ON HIV REPLICATION

The effects of IL-18 on HIV replication remain controversial (see ref [17] for a review). The cytokine has been shown to enhance HIV replication *in vitro* in chronically infected human monocytic and T cell lines [92, 93] as well as in an acutely infected T cell line [94]. The increased viral replication in the monocytic cell line was ascribed to the cytokine-induced activation of NF-κB and p38 MAPK, and involved IL-6 and TNF-α [92, 94]. Contrary to the results from these studies, one group of researchers reported that the cytokine inhibits, and its antagonist IL-18BP promotes HIV replication in human PBMC. The inhibition of the viral replication was reportedly due to increased production of IFN-γ in the cell cultures [95]. The authors also documented a decrease in the expression of CD4 in IL-18-treated PBMC cultures. Although IL-18 could potentially decrease HIV replication *via* inducing IFN-γ from NK and T cells, it most likely does not happen in these persons. In a relatively more recent study [75], the researchers noted a close association between IL-18 and IFN-γ levels of whole blood after stimulation with LPS in HIV-seronegative subjects. Despite observing enhanced levels of IL-18 in the whole blood in HIV-infected persons, they unexpectedly observed decreased levels of IFN-γ in the circulation of these patients as compared with those in HIV-seronegative healthy control subjects. These observations suggest that increased IL-18 concentrations in the circulation of HIV-infected persons do not result in enhanced IFN-γ levels. The probable reasons could be decreased concentrations of IL-12, IL-21 and IL-15, and increased production of TGF-β1 and IL-10. Therefore, IL-18-mediated inhibition of HIV replication *via* this interferon may be compromised in HIV-infected persons. The authors [75] have also observed that increased IL-18 levels correlated with decreased expression of CXCR4 on the patients' PBMC. The authors suggest an antiviral role of IL-18 due to its association with a decreased expression of the viral co-receptor CXCR4. However there is no direct experimental proof of the ability of IL-18 to decrease the expression of CXCR4 on human cells. It is noteworthy that in our hands recombinant human (rh) IL-18 increases HIV replication *in vitro* in purified human $CD4^+$ T cells (our unpublished data). In animal models of Rhesus macaques and Cynomolgous monkeys, pathogenic, but not non-pathogenic chimeric SIV/HIV-1 (SHIV) viruses caused increased IL-18 levels, which were associated with more rapid disease progression (loss of $CD4^+$ T cells), and a higher viral load set point [96]. Thus increased IL-18 concentrations *in vivo* seem to contribute towards disease progression. Nevertheless, it has been argued [97] that these increased levels of the cytokine represent a protective host response. They argue that neutralizing this IL-18 could have worsened the disease condition in these animals. Given that this response does not protect the infected animals, a counterargument that neutralizing IL-18 may benefit the host would seem more logical. Furthermore, in HIV-infected NOD-SCID mice, IL-18 administration increased HIV replication [98]. Taken together, it would be reasonable to conclude that raised IL-18 concentrations in HIV-infected persons contribute towards enhanced HIV replication. In line with this conclusion, raised serum levels of IL-18 in HIV-infected subjects have been associated with AIDS progression. The levels are higher in progressors but tend to remain stable in non-progressors [74]. In this regard anti-retroviral therapy, especially HAART, decreases the cytokine concentrations. However the levels always remain above normal and do not correlate with $CD4^+$ T cell counts [74]. It may be interesting to note that recombinant infectious clones of SIV that carried the simian IL-18 gene in the sense or anti-sense orientations had no effect on any virological or immunological parameters when injected in Rhesus macaques [99]. The clone with the IL-18 gene in the sense orientation induced only a slight increase in serum IFN-γ levels in early phase of the infection. No effect of the IL-18 gene expression was observed on viral replication [99]. Therefore, the collective evidence suggests that increased IL-18 concentrations in the sera of HIV-infected persons may not inhibit HIV replication; they may rather enhance it both directly in combination with other immunosuppressive cytokines, and indirectly by blunting antiviral immunity.

## ROLE OF IL-18 IN HIV-ASSOCIATED LIPODYSTROPHIC SYNDROME (HALS)

***What is HALS:*** First described in 1998 [100], the syndrome is characterized by fat redistribution, dyslipidemia, hypercholesterolemia, hyperlactatemia and systemic insulin resistance. The patients usually lose subcutaneous fat on face ("empty cheek syndrome"), arms, legs and upper trunk, and accumulate around viscera, breast and at ectopic locations, e.g., in the dorsocervical region giving rise to "buffalo hump" condition. It has been estimated that more than one third of the HIV-infected patients undergoing anti-retroviral therapy manifest HALS. The syndrome may also occur in the infected persons without receiving any therapy. However, anti-retroviral drugs especially reverse transcriptase inhibitors (RTI) and protease inhibitors (PI) hasten and exacerbate the condition ([101]; reviewed in [102]). Experimental studies have shown that wasting of the subcutaneous adipose tissue (lipoatrophy) occurs in this clinical condition due to inhibition of differentiation of pre-adipocytes as well as due to increased lipolysis and apoptosis in adipocytes [102, 103]. Fig. (**4**) shows how proinflammatory cytokines may interfere with the differentiation process of adipocytes. The number of cells undergoing apoptosis is markedly increased in the subcutaneous adipose tissue (SAT) of HALS patients. Increased lipolysis causes release of glycerol and non-esterified free fatty acids (FAA) that causes hyperlipidemia. FAAs increase insulin resistance (IR), induce production of proinflammatory mediators (e.g., IL-6 and TNF-α) from macrophages, and inhibit lipoprotein lipase (LPL) activity. They are also toxic to insulin-producing β-cells of pancreas [102].

The expression of the CCAAT/enhancer binding protein (C/EBP)-α and Peroxisome proliferator activated receptor (PPAR)-γ, which are necessary for adipogenesis, is reduced in lipoatrophic adipose tissue in HALS. The expression of the PPAR-γ co-activator (PGC)-1α, which regulates biogenesis of mitochondria, is also reduced in the adipose tissue in this syndrome. Furthermore, the respiratory chain in mitochondria of the adipose tissue becomes defective. Due

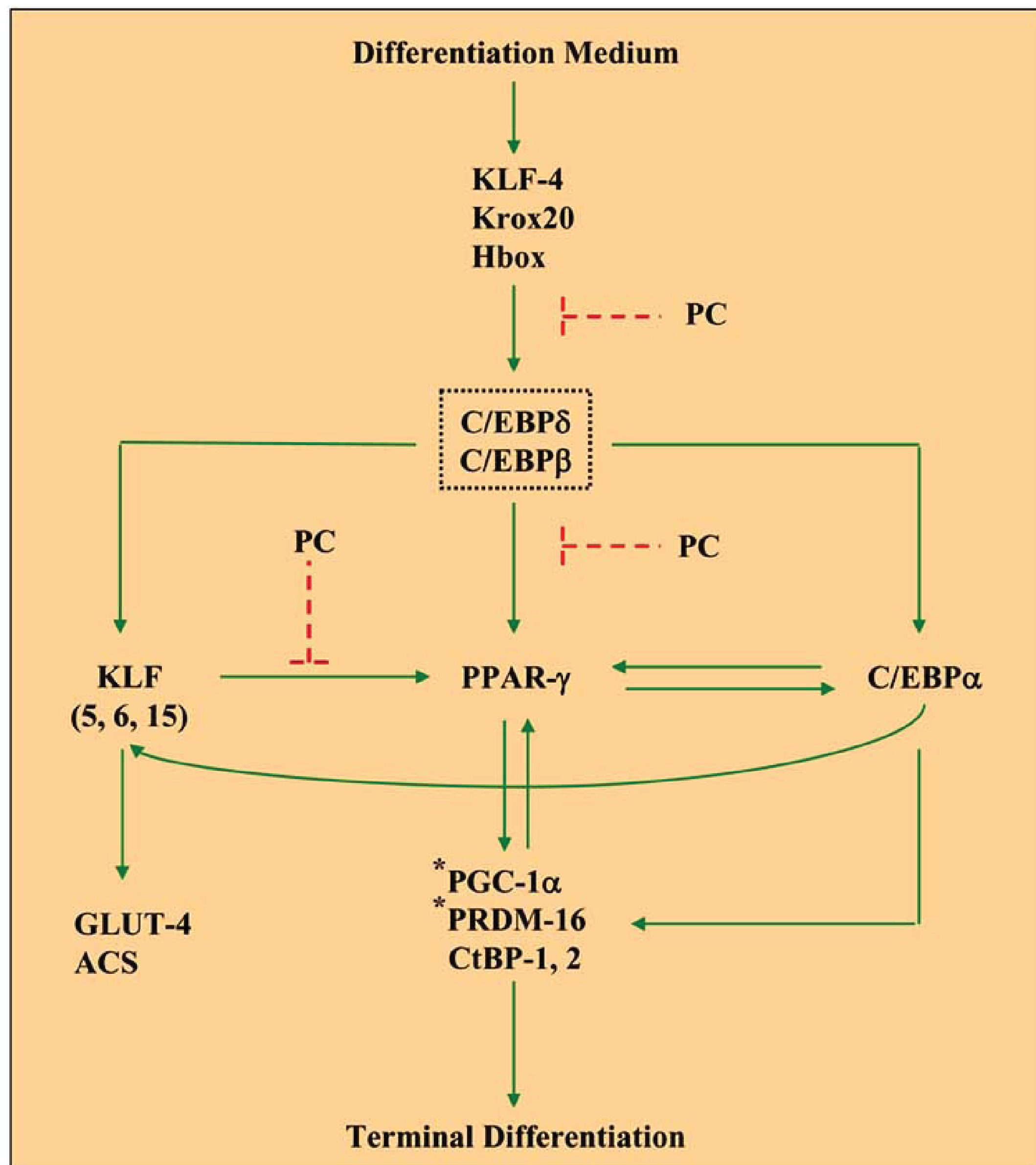


**Fig. (4). Adipocyte differentiation pathway.** The schematic figure depicts differentiation of 3T3-L1 preadipocytes after culturing them in the medium containing MID (a cocktail of differentiation-inducing mediators: methylisobutylxanthine, insulin and dexamethasone). Dashed lines indicate various steps at which proinflammatory cytokines inhibit the differentiation process. ACS: Acetyl co-enzyme A synthetase-2, CtBP: C-terminal binding protein, GLUT-4: Glucose transporter-4, Hbox: Homeobox transcription factor, KLF: Kruppel-like zinc finger factor, Krox-20: A zinc finger-containing transcription factor, PC: Proinflammatory cytokines like TNF-α, IL-6, etc., PGC-1α: PPAR-γ co-activator protein-1α, PRDM-16: PR domain containing protein-16. The genes shown in the box with a dotted line are expressed temporarily early in the differentiation process. An asterisk (*) indicates the factors known to mediate terminal differentiation of brown adipocytes.

to reduced expression of the uncoupling protein-2 (UCP-2; that controls ROS production), the mitochondria produce more reactive oxygen species (ROS), which cause oxidative stress to AT. ROS also induce increased production of proinflammatory mediators (see below).

Studies have also shown that accumulation of fat at ectopic places, e.g., dorsal region of neck (the "buffalo hump" condition), pubis and trunk, represents a distinct disturbance of adipose tissue. It results from accumulation of adipocytes, which express markers of brown adipocytes, e.g., smaller size, multilocularity, expression of UCP-1 and the proliferating cell nuclear antigen (PCNA; reviewed in [104]).

Brown adipocytes specialize in burning lipids for the production of heat. Each cell has many small lipid vacuoles (multilocular) and often expresses markers of cell proliferation (e.g., PCNA). The cells are rich in mitochondria, whose iron-rich proteins confer characteristic brown appearance to these cells. Another feature of these cells is expression of the mitochondrial uncoupling protein (UCP)-1 (also known as thermogenin), a proton transporter that short-circuits oxidative phosphorylation to energy production in the form of heat. Recent studies have demonstrated the existence of functional brown adipose tissue (BAT) in substantial amounts in the paracervical and supraclavicular regions in adult humans [105-107]. It is

noteworthy that these are the anatomical sites, where the "buffalo hump" anomaly develops in HALS patients. The "buffalo hump" adipose tissue, however, has lower levels of mitochondrial DNA and lacks local inflammatory response. It is not yet clear whether this tissue develops from a selective amplification of pre-existing brown adipocytes or results from a "browning" of white adipocytes (reviewed in [108]). There is some evidence to suggest that certain PI drugs and defective mitochondrial function may induce expression of UCP-1 and development of "buffalo hump" in HIV-infected persons [104]. In contrast to brown adipocytes, white adipocytes constitute bulk of adipose tissue in adult humans. These cells are specialized to store excess energy as lipids and produce a wide array of cytokines, chemokines and hormones often referred to as adipokines (see below) that affect functions of many other systems in the body. New adipocytes develop from the recruitment and differentiation of preadipocytes into fully differentiated adipocytes. The preadipocytes are precursors of fat cells and reside in vascular stroma of adipose tissues [109].

***Role of IL-18:*** It is noteworthy that IL-18 is one of several proinflammatory cytokines, whose concentrations are increased in the circulation as well as in the adipose tissues in HIV-infected HALS patients. The potential role of this cytokine in the etiopathogenesis of HALS is beginning to emerge. IL-18 mRNA and protein as well as IL-18R transcripts have been detected in the adipose tissue, including adipocytes, and TNF-α increases this expression [110]. As mentioned above, concentrations of IL-18 increase in the sera of HIV-infected persons. Interestingly, these levels are significantly higher in the patients with lipoatrophy as compared to those of the patients without it [111]. The levels correlate with those of TNF-α, but not with those of IL-6. Furthermore, an inverse correlation was found between IL-18 levels and the limb fat quantity, implicating this cytokine with lipoatrophy [111]. The level of IL-18 gene expression in the femoral gluteal adipose tissue correlated with the degree of lipoatrophy in these patients [112]. It was also observed that in the subcutaneous tissue, from where fat disappears, IL-18 gene expression is increased. In keeping with proapoptotic effects of the cytokine, an increased number of cells undergoing apoptosis were observed in adipose tissue aspirates obtained from the subcutaneous tissues of HIV-infected persons with HALS [113]. Taken together these studies suggest that IL-18 is implicated in the depletion of adipocytes from these areas. IL-18 also contributes towards HALS by downregulating the production of adiponectin. It inhibits transcription, mRNA expression as well as protein secretion from the adiponectin gene by phosphorylating NFAT-c4 *via* ERK1 and 2 [114]. Other proinflammatory cytokines such as TNF-α also exert similar effects on adiponectin [115]. Consequently, the concentrations of this important anti-inflammatory mediator, which is produced by adipocytes, decrease in HALS patients [116]. Adiponectin plays an important role in antagonizing the adverse effects of proinflammatory cytokines on HALS and insulin resistance (see below). Taken together these observations suggest that IL-18 plays an important role in the lipoatrophy seen in HALS patients.

Contrary to the role of IL-18 in lipoatrophy, its role in the ectopic accumulation of fat in HALS remains unknown. As mentioned above, the fat accumulated in the "buffalo hump" condition in HALS patients resembles with brown fat in certain features. It is noteworthy that brown adipocytes differ from white ones in their cellular origins as well as differentiation programs. The brown adipose tissue could be induced in response to cold and sympathetic nervous system activation [117]. It would be interesting to investigate the effects of the cytokine on the differentiation of brown adipocytes.

## HAART, IL-18 AND HALS

Seveal studies have shown that HAART inhibits HIV replication and reduces levels of serm IL-18 in HIV-infected persons. However, the levels persist at higher levels and never return to their physiological levels [16, 74]. The sustained higher levels of the cytokine continue to exert its effects on the body's adipose tissues. How HAART affects production of IL-18BP remains unknown. HAART often exacerbates HALS. Several drugs used in the HAART directly affect adipose tissue. For example, AZT is known to inhibit replication of mitochondrial DNA and biogenesis of mitochondria in adipocytes. Decreased numbers of mitochondria adversely affect functions of the adipose tissues [118]. Similarly, HIV protease inhibitors (PI) often cause cellular stress, inhibit the normal differentiation process of adipocytes and reduce fat storing capacity of the body [119]. Some of these drugs promote adipogenesis and induce expression of UCP-1; a feature of brown adipocytes [120]. These drugs are likely to promote lipohypertrophy in HALS. The drugs and the cytokine are likely to act in synergy to produce characteristic features of the "buffalo hump" fat in HIV-infected patients.

## IL-18 AND INSULIN RESISTANCE:

Production of proinflammatory cytokines from adipose tissues provides a link between obesity and metabolic disorders, e.g, insulin resistance (IR). In fact it is the inflammation induced by these cytokines rather than obesity *per se* that underlies obesity-associated IR, T2D and T2D-associated nephropathy and retinopathy [121, 122]. These cytokines may increase IR in many ways: They interfere with insulin-mediated signaling, induce expression of suppressors of cytokine signaling (SOCS) proteins that also increase insulin resistance, disturb adipocyte metabolism and decrease ability of the adipose tissue to store lipids. Consequently, there is dyslipidemia and lipids start accumulating in ectopic places, e.g., liver, pancreas, heart and skeletal muscles and increase insulin resistance in these organs. Increased IL-18 concentrations in HIV-infected individuals are likely to contribute to insulin resistance and metabolic disorders in all these fashions. Hypercholesterolemia, dyslipidemia (hypertriglyceridemia with lower levels of HDL and increased levels of LDL and VLDL), insulin resistance, increased fasting levels of glucose and insulin in the blood, were reported in HIV-infected persons even before the advent of HAART era [100, 123]. These manifestations occur early in the course of the infection and exacerbate with disease progression. Ironically certain HAART regimens may further contribute to their pathogenesis. The implication of IL-18 in these manifestations is supported by the reports that the serum levels of this cytokine are significantly higher in HIV-infected patients with hypertriglyceridemia as compared to

the patients with normal triglyceride levels [124]. It is noteworthy that increased concentrations of this cytokine correlate positively with serum triglyceride and glucose levels in obese and diabetic subjects [125]. They have also been reported in the circulation of HIV-seronegative T2D patients either diagnosed recently or with a long history of the disease. The levels decrease with high fiber diet [126]. Increased levels of the cytokine predict nephropathy in these patients [127]. Increased levels of IL-18 also correlate with obesity, glucose intolerance and predict the onset of T2D in obese patients. The levels decrease with weight loss in obese individuals ([126, 128-130]; reviewed in [131]). Interestingly, the cytokine and its receptor are expressed in pancreas in humans. The cytokine, alone as well as in combination with IL-12, causes acute pancreatitis and destruction of insulin-producing cells [132, 133]. Moreover, administration of exogenous IL-18 promotes development of diabetes in young NOD mice [134]. The cytokine is also responsible for streptozotocin-induced diabetes in mice, and IL-18BP alleviates the disease in these mice [135]. Taken together, it can be concluded that increased IL-18 levels in HIV-infected persons play a role in increasing their resistance to insulin.

## CARDIOVASCULAR (CV) DISEASES

IL-18 has been implicated in the development and progression of atherosclerosis. Functional IL-18Rs are expressed in atheroma-associated vascular endothelial cells and smooth muscles, which are augmented by LPS, TNF-α, IL-1β and other proinflammatory stimuli ([136]; reviewed in [22]). Mononuclear phagocytes in the lesions express mature IL-18. IL-18 induces several biological effects in the vascular endothelial cells that are reminiscent of atherogenesis: induction of IL-6, IL-8, ICAM-1 and various matrix metalloproteinases. Unexpectedly the cytokine also induces IFN-γ in mononuclear phagocytes and smooth muscle cells [136, 137]. It also induces expression of IFN-γ from aortic smooth muscle cells that, in turn, induce expression of CXCL16/SR-PSOX on macrophages [138]. The macrophages uptake oxidized LDL *via* the scavenger receptor SR-PSOX, whereas the secreted from of it (CXCL16) acts as a chemokine and attracts CXCL-16-positive T cells to the ldeveloping lesion. Increased levels of IL-18 expression in atherosclerotic plaques have been associated with plaque instability that may lead to acute ischemic syndromes [139]. Increased levels of the cytokine also occur in the circulation of atherosclerosis patients and they correlate with thickness of the intima-media of coronary artery [140, 141]. The thickness reflects overall burden of systemic atherosclerosis. Increased levels of the cytokine have also been reported in patients having unstable angina pectoris as well as in acute coronary disease [142, 143]. More importantly, *in vivo* neutralization of IL-18 by its natural antagonist (IL-18BP) slows down the development of atherosclerosis in a mouse model of the disease [144]. In a prospective study, Blankeberg *et al.* [145] have shown that increased IL-18 levels predict poor prognosis and increased risk of deaths in the patients with established coronary heart disease. IL-18 induces secretion of the plasminogen activator inhibitor (PAI-1) from adipocytes and promotes thrombogenic events [114]. Increased levels of this thrombogenic mediator in the circulation of HALS patients have been documented [146]. The IL-18-induced secretion of PAI-1 suggests that IL-18 may be causally involved in the enhanced platelet activation. Indeed, we have demonstrated a positive correlation between serum IL-18 levels and platelet activation in HIV-infected persons [79]. Heart failures with increased IL-18 concentrations in the circulation cause more deaths than the heart failures with physiological levels of the cytokine (reviewed in [147]). The cytokine is expressed in heart itself: many cell types in this organ e.g., cardiomyocytes, smooth muscle cells, endothelial cells, resident macrophages and infiltrated neutrophils, etc, produce this cytokine [147]. It causes death of cardiac endothelial cells by activating a novel signaling pathway, wich involves NF-κB and PTEN [148]. However, the cytokine induces hypertrophy in cardiomyocytes [149]. Interestingly, CRP, which is produced in liver in response to IL-6 and whose concentrations increase in the circulation in inflammatory conditions, induces IL-18 production from vascular endothelial cells [150].

More than 60% of the patients receiving anti-HIV drugs develop hyperlipidemia, hyperglycemia, and central obesity [151]. Several studies have documented occurrence of increased carotid intima media thickness and different biomarkers of cardiovascular risk (cholesterolemia, triglyceridemia, dyslipidemia, lower homocysteinemia, increased CRP levels, etc) in HIV-infected children and adults as compared to their HIV-seronegative counterparts [152-154]. A case controlled study described an increased occurrence of atherosclerotic plaques in HIV-positive patients [151]. Occurrence of cardiovascular events is a real risk in HIV-infected patients undergoing ART [155]. Use of lipid-lowering treatments may have been useful in stabilising this risk. It is highly likely that increased IL-18 concentrations in the circulation of in these patients play a role in the development of this risk. Neutralizing these concentrations with IL-18BP could prove to be a beneficial strategy in these patients.

## HIV-ASSOCIATED DEMENTIA (HAD)

HAD is a severe neurological complication in HIV-infected persons. It is characterized by development of severe cognitive, behavioral and motor dysfunctions (reviewed in [156, 157]). In the brain, both HIV-infected and non-infected macrophages and microglia produce proinflammatory cytokines like TNF-α, IL-1β and IL-18. These proinflammatory cytokines are believed to play a major role in the pathogenesis of HAD (reviewed in [28, 158]). These cytokines increase permeability of the blood-brain barrier that allows infiltration of HIV-infected macrophages into brain. The cytokines cause neuronal death by a variety of mechanisms, e.g., by overstimulating N-methyl-D-aspartate (NMDA) receptors, and inducing NO and reactive oxygen and nitrogen species (RONS). Increased concentrations of IL-18 occur in the circulation and cerebrospinal fluid (CSF) in patients suffering from psychic disorders [158]. It is noteworthy that the viral envelope glycoprotein Gp120 induces caspase-1 activation and IL-1β secretion from microglia and astrocytes in the brain [158]. These cells also express IL-18 and are likely to produce this cytokine in response to HIV infection. Indeed, increased levels of IL-18 occur in the CSF of HIV-infected persons suffering from opportunistic infections of the central nervous

system [159]. It is noteworthy that a significant correlation was found between IL-18 production and cognitive decline in Alzheimer disease patients [160]. Given that HIV-infected patients have increased concentrations of biologically active IL-18, the cytokine is likely to contribute towards the development of HAD in these patients.

## HIV AND IL-18 GENE POLYMORPHISM

The human IL-18 gene maps to chromosome 11q22.2 to 11q23.3. It contains 6 exons and 5 introns spread over 20.8 kb region. Two TATA-less promoters, located upstream exons 1 (Promoter 1) and 2 (Promoter 2), have been described for the gene. The promoter 1 is constitutively active in cell lines like THP-1 and U937, while the promoter 2 is induced by LPS and infectious agents [161]. It has been shown that promoter 1 is exclusively used in adrenal gland constitutively as well as in response to neurogenic stimuli, while promoter 2 is used by cells of the immune system [162]. Despite frequent sequencing of the gene in diverse human populations, no non-synonymous SNP could be found in the exons, and exon-intron boundaries of the gene. Furthermore, no SNP could be detected in the mRNA splice sites. A limited degree of polymorphism has been reported in the promoter and 3′ and 5′ untranslated regions (UTR) of the IL-18 gene (reviewed in [163]). Three synonymous SNP have been described in the promoter region of the IL-18 gene: G-656T, C-607A and G-137A. Of these SNP, replacing C at -607 with A abrogates a cAMP response element-binding site, whereas replacing G at -137 with C abrogates a DNA binding site for the nuclear factor H4TF1. The haplotype CG is associated with an increased production of IL-18 protein [164]. Interestingly, -607C was recently shown to be a risk factor for vertical transmission of HIV infection in a cohort of Brazillian children born to HIV-infected mothers, who took no antiretroviral therapy during pregnancy and did not undergo Caesarian section [165]. This study suggests that the children with genetic ability to produce more IL-18 may be at a higher risk of perinatal infection. However, it is noteworthy that –607C alone has not been associated with increased production of the cytokine. It would have been interesting to determine the IL-18 promoter genotype of the mothers. It is quite likely that the mothers may have a high IL-18 producer genotype of their IL-18 promoter. Such mothers may be simply having higher viral loads and hence may transmit HIV to their newborns more efficiently.

Several studies have reported varying degrees of association of IL-18 gene SNP and their haplotypes with atherosclerosis, multiple sclerosis, coronary heart disease, acute graft versus host disease, Crohn's disease and ulcerative colitis. Unfortunately, no clear-cut correlations could be demonstrated between predisposing genotypes and enhanced IL-18 production. Furthermore, many of the results have been contradicted by other studies (reviewed in [163]). As mentioned above, the biological activity of this cytokine is mainly controlled by IL-18BP and activation of caspase-1. The genetic variations that lead to a diminished production of IL-18BP and/or enhanced activation of caspase-1 could also result in enhanced biological activities of this proinflammatory cytokine, and hence may predispose humans to proinflammatory diseases. They may confound the effects of the genetic polymorphism in IL-18 gene on biological activities of the cytokine and their associations with different disease conditions.

## CONCLUSIONS AND FUTURE DIRECTIONS

The fact that certain viruses have incorporated the genes for IL-18BP and ICE inhibitor (e.g., the Cytokine Response Modifier gene in certain Pox viruses) into their genomes suggests the importance of IL-18 and related cytokines as an impediment to viral replication. However, HIV has probably developed alternate strategies to evade antiviral effects of this cytokine. It compromises the production of other cytokines (e.g., IL-12, IL-15, IL-21, etc) without which IL-18-induced responses blunt antiviral immune responses. Furthermore, increased production of immunosuppressive cytokines like TGF-β1 and IL-10 attenuates IL-18-induced IFN-γ production. Indeed, a developing body of data collected from clinical studies suggests that increasing concentrations of biologically active IL-18 in the circulation of HIV-infected individuals undermine innate and adaptive antiviral immune responses, especially in later stages of the infection. They may also enhance HIV replication in combination with other cytokines. Because of its ability to induce other inflammatory cytokines and amplify TH17 type $CD4^+$ effector/memory T cells, it might be involved in tissue destruction in this infection.

IL-18 is a cytokine produced by adipose tissue and it regulates lipid metabolism, energy homeostasis, insulin resistance and cardiovascular manifestations. It is noteworthy that studies in IL-18 and in IL-18R KO mice suggest a protective role of this cytokine in obesity, as these mice develop obesity, insulin resistance and atherosclerotic lesions with age [166]. They also exhibit defective control of food intake by the hypothalamic satiety center and become hyperphagic. The plasma IL-6 levels are not affected in these mice. In accord with these results, IL-18BP-over-expressing mice also overeat and become hyperglycemic. IL-18 deficiency caused decreased levels of constitutively activated STAT-3 in these mice [166]. Interestingly, intracerebral but not intravenous or intraperitoneal injections of recombinant murine IL-18 reversed these effects [166]. These data suggest that effects of IL-18 on insulin resistance and adiposity may be quite different depending upon its route of administration: it may promote insulin sensitivity and energy expenditure when acting on brain and may promote adiposity and insulin resistance when acting in the periphery (organs). Similar results have been reported for another proinflammatory cytokine IL-6. IL-6 KO mice develop mature-onset obesity, IR and diabetes. Its peripheral administration promotes insulin resistance and dyslipidemia, whereas its administration into CNS protects mice from these manifestations [127, 167]. Clearly further studies are needed to learn more on the effects of IL-18 and its natural antagonist on energy and lipid homeostasis in animal models. Furthermore, while IL-18 gene knocked-out and transgenic mice provide valuable information, such studies must be verified by neutralization of the cytokine by siRNA or antagonist to avoid potential effects of compensatory mechanisms. It may be relevant to mention here that it has been argued that obese individuals as well as T2D patients develop resistance to IL-18, as their leukocytes respond

poorly to this cytokine in *ex vivo* studies due to reduced expression of IL-18Rα and β chains on their surface [168]. Due to a compromised production of IL-12, which induces expression of IL-18Rβ on naïve T cells, it is highly likely that the number of T cells responding to IL-18 may decline especially in later stages in the course of HIV infection. Unfortunately no studies have been reported on the expression of IL-18R or responsiveness of human cells to this cytokine in HIV-infected cells. Such studies are highly desirable for fully understanding the role of this cytokine in controlling HIV infection and its role in antiviral immunity and immunopathogenesis.

In the context of HIV infection, increased concentrations of IL-18 blunt antiviral immunity in HIV-infected patients, contribute towards immunopathogenesis of AIDS and promote disturbances in fat metabolism, insulin resistance, atherogenesis and cardiovascular manifestations (see Table **2**). Therefore targeting IL-18 may be a therapeutic option. Fortunately, a natural antagonist with strong neutralizing activity exists for this cytokine and may be the best option. Moreover, small molecular inhibitors of ICE have been developed. They inhibit ICE-mediated processing of IL-18 and IL-1β precursors into their biologically active forms. They may be used to reduce activities of these proinflammatory cytokines in chronic infectious and inflammatory conditions. Still P2X7R may be another target. Small molecular inhibitors of the receptor may be used to modulate inflammatory responses mediated by IL-18 and related cytokines (IL-1β and IL-33). These therapeutic options may be exercised in HIV-infected patients under close monitoring for unpredictable side effects.

**Table 2. How IL-18 Contributes Towards AIDS Pathogenesis**

1. It promotes TH2 type immune responses *via* its actions on basophils and mast cells [66].
2. It modulates amplification and differentiation of TH17 cells and may disturb their balance with Treg cells [61, 186].
3. It enhances HIV replication especially in concert with other cytokines [92-94].
4. It causes NK cell loss by fratricidal killing of NK cells *via* FasL and TNF-α [15].
5. It promotes the development of HALS and other metabolic disorders in HIV-infected patients [112, 124].
6. It promotes HIV-associated dementia by interfering with brain cell functions [159, 187].

Proinflammatory cytokines are known to regulate fat metabolism, induce IR and promote atherogenic process. They mediate some of these effects by their direct effects on adipocytes. Little is known about potential effects of IL-18 on adipocytes and insulin-mediated signaling in human cells. It is noteworthy that human adipocytes express this cytokine and its receptor as well. Future studies should address these issues. Given that brown and white adipocytes arise from distinct precursors and undergo distinct programs of differentiation, such studies should be conducted on both types of cells separately. A potential role of IL-18 or its antagonist in the differential behavior of the subcutaneous and visceral adipose tissues also needs to be carefully examined. The results may be important for understanding the etiopathogenesis of HALS. They will also have implications for obesity, diabetes and atherosclerosis.

Experimental studies have shown that IFN-γ induces IL-12Rβ1 chain on naïve $CD4^+$ T cells in an IRF-1 dependent pathway that is required for IL-12-mediated differentiation of these cells into TH1 type cells. An early production of IFN-γ, therefore, is important for inducing effective antiviral immune responses. By its ability to induce this cytokine, IL-18 could serve as an effective adjuvant for anti-HIV vaccination. In vaccination studies, the injection of IL-18-encoding DNA as an adjuvant has been shown to induce protective TH1 type immunity. In this regard, co-injection of IL-18 and IL-12 encoding DNA may be more effective (reviewed in [54]). Future studies on anti-HIV vaccination using this cytokine as an adjuvant are highly desirable.

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## REFERENCES

[1] Thompson AJV, Locarnini SA. Toll-like receptors, RIG-I-like RNA helicases and the antiviral innate immune response. Immunol Cell Biol 2007; 85(6): 435-45.

[2] Trinchieri G, Sher A. Cooperation of Toll-like receptor signals in innate immune defence. Nat Rev Immunol 2007; 7(3): 179-90.

[3] Shaw MH, Reimer T, Kim Y-G, Nuoez G. NOD-like receptors (NLRs): bona fide intracellular microbial sensors. Curr Opin Immunol 2008; 20(4): 377-82.

[4] Sirard J-C, Vignal Cc, Dessein R, Chamaillard M. Nod-like receptors: Cytosolic watchdogs for immunity against pathogens. PLoS Pathogens 2007; 3(12): e152.

[5] Biron C, Cousens L, Ruzek M, Su H, Salazar-Mather T. Early cytokine responses to viral infections and their roles in shaping endogenous cellular immunity. Adv Exp Med Biol 1998; 452: 143-9.

[6] Estcourt M, Ramshaw A, Ramsay A. Cytokine responses in virus infections: effects on pathogenesis, recovery and persistence. Curr Opin Microbiol 1998; 1(4): 411-8.

[7] Fuse S, Molloy M, Usherwood E. Immune responses against persistent viral infections: possible avenues for immunotherapeutic interventions. Crit Rev Immunol 2008; 28(2): 159-83.

[8] Schroder M, Bowie AG. An arms race: innate antiviral responses and counteracting viral strategies. Biocheml Soc Trans 2007; 035(6): 1512-4.

[9] Shearer GM. HIV-induced immunopathogenesis. Immunity 1998; 9(5): 587-93.

[10] Kaiko G, Horvat J, Beagley K, Hansbro P. Immunological decision-making: how does the immune system decide to mount a helper T-cell response? Immunology 2008; 123(3): 326-38.

[11] Ahmad A, Ahmad R, Toma E, Morisset R, Menezes J. Impaired induction of IL-15 in response to herpes simplex virus type 1 infection in peripheral blood mononuclear cells of HIV-infected patients. AIDS 2000; 14(6): 744-6.

[12] Chambers KA, Parato KG, Angel JB. Active cellular infection of myeloid cells is required for HIV-1-mediated suppression of interleukin-12 p40 expression. Cell Immunol 2002; 215(2): 120-32.

[13] Chehimi J, Starr S, Frank I, *et al*. Impaired interleukin 12 production in human immunodeficiency virus-infected patients. J Exp Med 1994; 179(4): 1361-6.

[14] Iannello A, Tremblay C, Routy J, Boulassel M, Toma E, Ahmad A. Decreased levels of circulating IL-21 in HIV-infected AIDS patients: Correlation with CD4+ T cell counts. Viral Immunol 2008; 21(3): 385-8.

[15] Iannello A, Debbeche O, Samarani S, Ahmad A. Antiviral NK cell responses in HIV infection: II. viral strategies for evasion and

lessons for immunotherapy and vaccination. J Leukoc Biol 2008; 84(1): 27-49.

[16] Ahmad R, Sindhu STA, Toma E, Morisset R, Ahmad A. Elevated levels of circulating interleukin-18 in human immunodeficiency virus-infected individuals: Role of peripheral blood mononuclear cells and implications for AIDS pathogenesis. J Virol 2002; 76(24): 12448-56.

[17] Torre D, Pugliese A. Interleukin-18: a proinflammatory cytokine in HIV-1 infection. Curr HIV Res 2006; 4(4): 423-30.

[18] Nakamura K, Okamura H, Wada M, Nagata K, Tamura T. Endotoxin-induced serum factor that stimulates gamma interferon production. Infect Immun 1989; 57(2): 590-5.

[19] Okamura H, Nagata K, Komatsu T, *et al*. A novel costimulatory factor for gamma interferon induction found in the livers of mice causes endotoxic shock. Infect Immun 1995; 63(10): 3966-72.

[20] Okamura H, Tsutsui H, Komatsu T, *et al*. Cloning of a new cytokine that induces IFN-[gamma] production by T cells. Nature 1995; 378(6552): 88-91.

[21] Biet F, Locht C, Kremer L. Immunoregulatory functions of interleukin 18 and its role in defense against bacterial pathogens. J Mol Med 2002; 80(3): 147-62.

[22] Dinarello C. Interleukin-18 and the pathogenesis of inflammatory diseases. Semin Nephrol 2007; 27(1): 98-114.

[23] Sekiyama A, Ueda H, Kashiwamura S, *et al*. IL-18; a cytokine translates a stress into medical science. J Med Invest 2005; 52: 236-9.

[24] Gardella S, Andrei C, Costigliolo S, Poggi A, Zocchi MR, Rubartelli A. Interleukin-18 synthesis and secretion by dendritic cells are modulated by interaction with antigen-specific T cells. J Leukoc Biol 1999; 66(2): 237-41.

[25] Dinarello C. The IL-1 family and inflammatory diseases. Clin Exp Rheumatol 2002; 20(5): S1-13.

[26] Ghayur T, Banerjee S, Hugunin M, *et al*. Caspase-1 processes IFN-[gamma]-inducing factor and regulates LPS-induced IFN- [gamma] production. Nature 1997; 386(6625): 619-23.

[27] Gu Y, Kuida K, Tsutsui H, *et al*. Activation of interferon-gamma inducing factor mediated by interleukin-1beta converting enzyme. Science 1997; 275(5297): 206-9.

[28] Simi A, Lerouet D, Pinteaux E, Brough D. Mechanisms of regulation for interleukin-1[beta] in neurodegenerative disease. Neuropharmacology 2007; 52(8): 1563-9.

[29] Willingham SB, Ting JPY. NLRs and the dangers of pollution and aging. Nat Immunol 2008; 9(8): 831-3.

[30] Qu Y, Franchi L, Nunez G, Dubyak GR. Nonclassical IL-1beta secretion stimulated by P2X7 receptors is dependent on inflammasome activation and correlated with exosome release in murine macrophages. J Immunol 2007; 179(3): 1913-25.

[31] Ting J, Willingham S, Bergstralh D. NLRs at the intersection of cell death and immunity. Nat Rev Immunol 2008; 8(5): 372-9.

[32] Halle A, Hornung V, Petzold GC, *et al*. The NALP3 inflammasome is involved in the innate immune response to amyloid-[beta]. Nat Immunol 2008; 9(8): 857-65.

[33] Hewinson J, Moore SF, Glover C, Watts AG, MacKenzie AB. A key role for redox signaling in rapid P2X7 receptor-induced IL-1{beta} processing in human monocytes. J Immunol 2008; 180(12): 8410-20.

[34] Mariathasan S, Weiss DS, Newton K, *et al*. Cryopyrin activates the inflammasome in response to toxins and ATP. Nature 2006; 440(7081): 228-32.

[35] Ozoren N, Masumoto J, Franchi L, *et al*. Distinct roles of TLR2 and the adaptor ASC in IL-1beta/IL-18 secretion in response to Listeria monocytogenes. J Immunol 2006; 176(7): 4337-42.

[36] Perregaux DG, McNiff P, Laliberte R, Conklyn M, Gabel CA. ATP acts as an agonist to promote stimulus-induced secretion of IL-1{beta} and IL-18 in human blood. J Immunol 2000; 165(8): 4615-23.

[37] Li H, Ambade A, Re F. Cutting edge: Necrosis activates the NLRP3 inflammasome. J Immunol 2009; 183(3): 1528-32.

[38] Hsu L, Ali S, McGillivray S, *et al*. A NOD2-NALP1 complex mediates caspase-1-dependent IL-1beta secretion in response to Bacillus anthracis infection and muramyl dipeptide. PNAS 2008; 105(22): 7803-8.

[39] Dinarello C. Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. Int Rev Immunol 1998; 16(5-6): 457-99.

[40] Sims J. IL-1 and IL-18 receptors, and their extended family. Curr Opin Immunol 2002; 14(1): 117-22.

[41] Kato Z, Jee J, Shikano H, *et al*. The structure and binding mode of interleukin-18. Nat Struct Biol 2003; 10(11): 966-71.

[42] Kumar S, McDonnell PC, Lehr R, *et al*. Identification and initial characterization of four novel members of the interleukin-1 family. J Biol Chem 2000; 275(14): 10308-14.

[43] Novick D, Kim S-H, Fantuzzi G, Reznikov LL, Dinarello CA, Rubinstein M. Interleukin-18 binding protein: a novel modulator of the Th1 cytokine response. Immunity 1999; 10(1): 127-36.

[44] Hurgin V, Novick D, Rubinstein M. The promoter of IL-18 binding protein: activation by an IFN-gamma -induced complex of IFN regulatory factor 1 and CCAAT/enhancer binding protein beta. PNAS 2002; 99(26): 16957-62.

[45] Paulukat J, Bosmann M, Nold M, *et al*. Expression and release of IL-18 binding protein in response to IFN-{gamma}. J Immunol 2001; 167(12): 7038-43.

[46] Novick D, Schwartsburd B, Pinkus R, *et al*. A novel IL-18BP ELISA shows elevated serum IL-18BP in sepsis and extensive decrease of free IL-18 Cytokine 2001; 14(6): 334-42.

[47] Kim S-H, Eisenstein M, Reznikov L, *et al*. Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. PNAS 2000; 97(3): 1190-5.

[48] Reznikov L, Kim S, Westcott J, *et al*. IL-18 binding protein increases spontaneous and IL-1-induced prostaglandin production *via* inhibition of IFN-gamma. PNAS 2000; 97(5): 2174-9.

[49] Banda NK, Vondracek A, Kraus D, *et al*. Mechanisms of inhibition of collagen-induced arthritis by murine IL-18 binding protein. J Immunol 2003; 170(4): 2100-5.

[50] Reading PC, Smith GL. Vaccinia virus interleukin-18-binding protein promotes virulence by reducing gamma interferon production and natural killer and T-cell activity. J Virol 2003; 77(18): 9960-8.

[51] Xiang Y, Moss B. IL-18 binding and inhibition of interferon gamma induction by human poxvirus-encoded proteins. PNAS 1999; 96(20): 11537-42.

[52] Bufler P, Azam T, Gamboni-Robertson F, *et al*. A complex of the IL-1 homologue IL-1F7b and IL-18-binding protein reduces IL-18 activity. Proc Natl Acad Sci USA 2002; 99(21): 13723-8.

[53] Arend W, Palmer G, Gabay C. IL-1, IL-18, and IL-33 families of cytokines. Immunol Rev 2008; 223(1): 20-38.

[54] Gracie JA, Robertson SE, McInnes IB. Interleukin-18. J Leukoc Biol 2003; 73(2): 213-24.

[55] Hyodo Y, Matsui K, Hayashi N, *et al*. IL-18 up-regulates perforin-mediated NK activity without increasing perforin messenger RNA expression by binding to constitutively expressed IL-18 receptor. J Immunol 1999; 162(3): 1662-8.

[56] Cannetti CA, Leung BP, Culshaw S, McInnes IB, Cunha FQ, Liew FY. IL-18 Enhances Collagen-Induced Arthritis by Recruiting Neutrophils *Via* TNF-{alpha} and Leukotriene B4. J Immunol 2003; 171(2): 1009-15.

[57] Verri WJ, Cunha T, Ferreira S, *et al*. IL-15 mediates antigen-induced neutrophil migration by triggering IL-18 production. Eur J of Immunol 2007; 37(12): 3373-80.

[58] Gutzmer R, Langer K, Mommert S, Wittmann M, Kapp A, Werfel T. Human dendritic cells express the IL-18R and are chemoattracted to IL-18. J Immunol 2003; 171(12): 6363-71.

[59] Maxwell JR, Yadav R, Rossi RJ, *et al*. IL-18 bridges innate and adaptive immunity through IFN-{gamma} and the CD134 pathway. J Immunol 2006; 177(1): 234-45.

[60] Harrington LE, Hatton RD, Mangan PR, *et al*. Interleukin 17-producing CD4+ effector T cells develop *via* a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005; 6(11): 1123-32.

[61] Weaver CT, Hatton RD, Mangan PR, Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. Ann Rev Immunol 2007; 25(1): 821-52.

[62] Sedger LM, Shows DM, Blanton RA, *et al*. IFN-{gamma} mediates a novel antiviral activity through dynamic modulation of TRAIL and TRAIL receptor expression. J Immunol 1999; 163(2): 920-6.

[63] Reading PC, Whitney PG, Barr DP, *et al*. IL-18, but not IL-12, regulates NK cell activity following intranasal Herpes Simplex virus type 1 infection. J Immunol 2007; 179(5): 3214-21.

[64] Dinarello C. IL-18: A TH1-inducing, proinflammatory cytokine and new member of the IL-1 family. J Allergy Clin Immunol 1999; 103: 11-24.

[65] Yoshimoto T, Takeda K, Tanaka T, *et al*. IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells, and B cells: Synergism with IL-18 for IFN-{gamma} production. J Immunol 1998; 161(7): 3400-7.

[66] Yoshimoto T, Nakanishi K. Roles of IL-18 in basophils and mast cells. Allergol Int 2006; 55(2): 105-13.

[67] Leite-de-Moraes MC, Hameg A, Pacilio M, *et al*. IL-18 enhances IL-4 production by ligand-activated NKT lymphocytes: A Pro-Th2 effect of IL-18 exerted through NKT cells. J Immunol 2001; 166(2): 945-51.

[68] Uchida T, Kinoshita M, Fukasawa M, Habu Y, Shinomiya N, Seki S. IL-18 time-dependently modulates Th1/Th2 cytokine production by ligand-activated NKT cells. Europ J Immunol 2007; 37(4): 966-77.

[69] Sugimoto T, Ishikawa Y, Yoshimoto T, Hayashi N, Fujimoto J, Nakanishi K. Interleukin 18 acts on memory T helper cells type 1 to induce airway inflammation and hyperresponsiveness in a naive host mouse. J Exp Med 2004; 199(4): 535-45.

[70] Pirhonen J. Regulation of IL-18 expression in virus infection. Scandinavian J Immunol 2001; 53(6): 533-9.

[71] Sugawara I. Interleukin-18 (IL-18) and infectious diseases, with special emphasis on diseases induced by intracellular pathogens. Microb Infect 2000; 2(10): 1257-63.

[72] Giavedoni LD, Velasquillo MC, Parodi LM, Hubbard GB, Hodara VL. Cytokine expression, Natural Killer cell activation, and phenotypic changes in lymphoid cells from rhesus macaques during acute infection with pathogenic Simian Immunodeficiency Virus. J Virol 2000; 74(4): 1648-57.

[73] Torre D, Speranza F, Martegani R, *et al*. Circulating levels of IL-18 in adult and paediatric patients with HIV-1 infection. AIDS 2000; 14(14): 2211-2.

[74] Stylianou E, Bjerkeli V, Yndestad A, *et al*. Raised serum levels of interleukin-18 is associated with disease progression and may contribute to virological treatment failure in HIV-1-infected patients. Clin Exp Immunol 2003; 132(3): 462-6.

[75] Sailer CA, Pott GB, Dinarello CA, *et al*. Whole-blood interleukin-18 level during early HIV-1 infection is associated with reduced CXCR4 coreceptor expression and interferon-[gamma] levels. J Infect Dis 2007; 195(5): 734-8.

[76] David D, Chevrier D, Treilhou M, *et al*. IL-18 underexpression reduces IL-2 levels during HIV infection: a critical step towards the faulty cell-mediated immunity? AIDS 2000; 14(14): 2212-4.

[77] He L, Terunuma H, Hanabusa H, *et al*. Interleukin 18 and interleukin 1beta production is decreased in HIV type 1-seropositive hemophiliacs but not in HIV type 1-seropositive nonhemophiliacs. AIDS Res Human Retroviruses 2000; 16(4): 345-53.

[78] Chaisavaneeyakorn S, Moore JM, Otieno J, *et al*. Immunity to placental malaria. III. Impairment of interleukin(IL)-12, not IL-18, and interferon-inducible protein-10 responses in the placental intervillous blood of human immunodeficiency virus/malaria-coinfected women. J Infect Dis 2002; 185(1): 127-31.

[79] Ahmad R, Iannello A, Samarani S, *et al*. Contribution of platelet activation to plasma IL-18 concentrations in HIV-infected AIDS patients. AIDS 2006; 20(14): 1907-9.

[80] Pizarro TT, Michie MH, Bentz M, *et al*. IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: Expression and localization in intestinal mucosal cells. J Immunol 1999; 162(11): 6829-35.

[81] Dandekar S. Pathogenesis of HIV in the gastrointestinal tract. Curr HIV/AIDS Rep 2007; 4(1): 10-5.

[82] Iannello A, Samarani S, Debbeche O, *et al*. Role of IL-18 in the development and pathogenesis of AIDS. AIDS Rev 2009; in press.

[83] Clerici M, Hakim F, Venzon D, *et al*. Changes in interleukin-2 and interleukin-4 production in asymptomatic, human immunodeficiency virus-seropositive individuals. J Clin Invest 1993; 91(3): 759-65.

[84] Meyaard L, Hovenkamp E, Keet IP, *et al*. Single cell analysis of IL-4 and IFN-gamma production by T cells from HIV-infected individuals: decreased IFN-gamma in the presence of preserved IL-4 production. J Immunol 1996; 157(6): 2712-8.

[85] Leach S, Messina I, Lemberg D, Novick D, Rubenstein M, Day A. Local and systemic interleukin-18 and interleukin-18-binding protein in children with inflammatory bowel disease. Inflammatory Bowel Diseases 2008; 14(1): 68-74.

[86] Liang D, Ma W, Yao C, Liu H, Chen X. Imbalance of interleukin 18 and interleukin 18 binding protein in patients with lupus nephritis. Cell Mol Immunol 2006; 3(4): 303-6.

[87] Ludwiczek O, Kaser A, Novick D, *et al*. Plasma levels of interleukin-18 and interleukin-18 binding protein are elevated in patients with chronic liver disease. J Clin Immunol 2002; 22(6): 331-7.

[88] Mazodier K, Marin V, Novick D, *et al*. Severe imbalance of IL-18/IL-18BP in patients with secondary hemophagocytic syndrome. Blood 2005; 106(10): 3483-9.

[89] Shearer G, Clerici M. Cytokine profiles in HIV type 1 disease and protection. AIDS Res Hum Retrovir 1998; 14: 149-52.

[90] Shibatomi K, Ida K, Yamasaki S, *et al*. A novel role for interleukin-18 in human natural killer cell death: High serum levels and low natural killer cell numbers in patients with systemic autoimmune diseases. Arthritis Rheum 2001; 44(4): 884-92.

[91] Iannello A, Samarani S, Debbeche O, *et al*. Potential role of IL-18 in the immunopathogenesis of AIDS: Involvement in fratricidal killing of NK cells. J Virol 2009; 83(12): 5999-6010.

[92] Pugliese A, Gennero L, Vidotto V, Speranza F, Tambini R, Torre D. Interleukin-18 enhances HIV-1 production in a human chronically-infected T cell line (H9-V). Cell Biochem Funct 2002; 20(4): 333-7.

[93] Shapiro L, Puren A, Barton H, *et al*. Interleukin 18 stimulates HIV type 1 in monocytic cells. PNAS 1998; 95(21): 12550-5.

[94] Klein S, Klebba C, Kauschat D, *et al*. Interleukin-18 stimulates HIV-1 replication in a T-cell line. Eur Cytokine Netw 2000; 11(1): 47-52.

[95] Choi HJ, Dinarello CA, Shapiro L. Interleukin-18 inhibits human immunodeficiency virus type 1 production in peripheral blood mononuclear cells. J Infect Dis 2001; 184(5): 560-8.

[96] Kaizu M, Ami Y, Nakasone T, *et al*. Higher levels of IL-18 circulate during primary infection of monkeys with a pathogenic SHIV than with a nonpathogenic SHIV. Virology 2003; 313(1): 8-12.

[97] Torre D. Is interleukin-18 a proviral or antiviral cytokine in HIV-1 infection? J Infect Dis 2007; 196(5): 803-4.

[98] Senpuku H, Asano T, Salam A, *et al*. Effects of human interleukin-18 and interleukin-12 treatment on human lymphocyte engraftment in NOD-scid mouse. Immunology 2002; 107(2): 232-42.

[99] Giavedoni LD, Velasquillo MC, Parodi LM, Hubbard GB, Hodara VL. Expression of IL-18 by SIV does not modify the outcome of the antiviral immune response. Virology 2002; 303(2): 327-37.

[100] Carr A, Samaras K, Burton S, *et al*. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. AIDS. 1998; 12(7): F51-8.

[101] Miller J, Carr A, Emery S, *et al*. HIV lipodystrophy: prevalence, severity and correlates of risk in Australia. HIV Med 2003; 4(3): 293-301.

[102] Villarroya F, Domingo P, Giralt M. Lipodystrophy in HIV 1-infected patients: lessons for obesity research. Int J Obes 2007; 31(12): 1763-9.

[103] Kotler DP, Ionescu G, Johnson JA, *et al*. Studies of adipose tissue metabolism in human immunodeficiency virus and associated lipodystrophy. Clin Infect Dis 2003; 37(s2): S47-S51.

[104] Guallar J, Gallego-Escuredo J, Domingo J, *et al*. Differential gene expression indicates that 'buffalo hump' is a distinct adipose tissue disturbance in HIV-1-associated lipodystrophy. AIDS 2008; 22(5): 575-84.

[105] Cypess AM, Lehman S, Williams G, *et al*. Identification and importance of brown adipose tissue in adult humans. N Engl J Med 2009; 360(15): 1509-17.

[106] van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, *et al*. Cold-activated brown adipose tissue in healthy men. N Engl J Med 2009; 360(15): 1500-8.

[107] Virtanen KA, Lidell ME, Orava J, *et al*. Functional brown adipose tissue in healthy adults. N Engl J Med 2009; 360(15): 1518-25.

[108] Cannon B, Nedergaard J. Brown adipose tissue: Function and physiological significance. Physiol Rev 2004; 84(1): 277-359.

[109] Balwierz A, Czech U, Polus A, *et al*. Human adipose tissue stromal vascular fraction cells differentiate depending on distinct types of media. Cell Prolif 2008; 41(3): 441-59.

[110] Wood IS, Wang B, Jenkins JR, Trayhurn P. The pro-inflammatory cytokine IL-18 is expressed in human adipose tissue and strongly upregulated by TNF[alpha] in human adipocytes. Biochem Biophys Res Commun 2005; 337(2): 422-9.

[111] Lindegaard B, Hansen A, Gerstoft J, Pedersen B. High plasma level of interleukin-18 in HIV-infected subjects with lipodystrophy. J Acquir Immune Defic Syndr 2004; 36(1): 588-93.

[112] Lindegaard B, Hansen AB, Pilegaard H, Keller P, Gerstoft J, BK P. Adipose tissue expression of IL-18 and HIV-associated lipodystrophy. AIDS 2004; 18(14): 1956-8.

[113] Domingo P, Matias-Guiu X, Pujol R, *et al*. Subcutaneous adipocyte apoptosis in HIV-1 protease inhibitor-associated lipodystrophy. AIDS 1999; 13(16): 2261-7.

[114] Chandrasekar B, Patel DN, Mummidi S, Kim J-w, Clark RA, Valente AJ. Interleukin-18 suppresses adiponectin expression in 3T3-L1 adipocytes *via* a novel signal transduction pathway involving ERK1/2-dependent NFATc4 phosphorylation. J Biol Chem 2008; 283(7): 4200-9.

[115] Zappalà G, Rechler M. IGFBP-3, hypoxia and TNF-alpha inhibit adiponectin transcription. Biochem Biophys Res Commun 2009; 382(4): 785-9.

[116] Addy CL, Gavrila A, Tsiodras S, Brodovicz K, Karchmer AW, Mantzoros CS. Hypoadiponectinemia is associated with insulin resistance, hypertriglyceridemia, and fat redistribution in human immunodeficiency virus-infected patients treated with highly active antiretroviral therapy. J Clin Endocrinol Metab 2003; 88(2): 627-36.

[117] Fruhbeck G, Becerril S, Sinz N, Garrastachu P, GarcÌa-Velloso MJ. BAT: a new target for human obesity? Trends Pharmacol Sci 2009; 30(8): 387-96.

[118] Deveaud C, Beauvoit B, Reynaud A, Bonnet J. Site-specific reduction of oxidative and lipid metabolism in adipose tissue of 3'-azido-3'-deoxythymidine-treated rats. Antimicrob Agents Chemother 2007; 51(2): 583-90.

[119] Flint OP, Noor MA, Hruz PW, *et al*. The role of protease inhibitors in the pathogenesis of HIV-associated lipodystrophy: Cellular mechanisms and clinical implications. Toxicol Pathol 2009; 37(1): 65-77.

[120] Rodriguez de la Concepcion M, Yubero P, Domingo J, *et al*. Reverse transcriptase inhibitors alter uncoupling protein-1 and mitochondrial biogenesis in brown adipocytes. Antivir Ther 2005; 10(4): 515-26.

[121] Herder C, Schneitler S, Rathmann W, *et al*. Low-grade inflammation, obesity, and insulin resistance in adolescents. J Clin Endocrinol Metab 2007; 92(12): 4569-74.

[122] Medina-Gomez G, Gray S, Vidal-Puig A. Adipogenesis and lipotoxicity: role of peroxisome proliferator-activated receptor gamma (PPARgamma) and PPARgammacoactivator-1 (PGC1). Public Health Nutr 2007; 10(10A): 1132-7.

[123] Hadigan C, Miller K, Corcoran C, Anderson E, Basgoz N, Grinspoon S. Fasting hyperinsulinemia and changes in regional body composition in human immunodeficiency virus-infected women. J Clin Endocrinol Metab 1999; 84(6): 1932-7.

[124] Falasca K, Manigrasso MR, Racciatti D, *et al*. Associations between hypertriglyceridemia and serum ghrelin, adiponectin, and IL-18 levels in HIV-infected patients. Ann Clin Lab Sci 2006; 36(1): 59-66.

[125] Olusi S, Al-Awadhi A, Abraham M. Relations of serum interleukin 18 levels to serum lipid and glucose concentrations in an apparently healthy adult population. Horm Res 2003; 60(1): 29-33.

[126] Ruderman NB, Keller C, Richard AM, *et al*. Interleukin-6 regulation of AMP-activated protein kinase. Potential role in the systemic response to exercise and prevention of the metabolic syndrome. Diabetes 2006; 55: S48-54.

[127] Araki S, Haneda M, Koya D, *et al*. Predictive impact of elevated serum level of IL-18 for early renal dysfunction in type 2 diabetes: an observational follow-up study. Diabetologia 2007; 50(4): 867-73.

[128] Thorand B, Kolb H, Baumert J, *et al*. Elevated levels of interleukin-18 predict the development of type 2 diabetes: results from the MONICA/KORA Augsburg study, 1984-2002. Diabetes 2005; 54(10): 2932-8.

[129] Esposito K, Pontillo A, Ciotola M, *et al*. Weight loss reduces interleukin-18 levels in obese women. J Clin Endocrinol Metab 2002; 87(8): 3864-6.

[130] Leick L, Lindegaard B, Stensvold D, Plomgaard P, Saltin B, Pilegaard H. Adipose tissue interleukin-18 mRNA and plasma interleukin-18: Effect of obesity and exercise. Obesity 2007; 15(2): 356-63.

[131] Falasca K, Ucciferri C, Manzoli L, *et al*. Metabolic syndrome and cardiovascular risk in HIV-infected patients with lipodystrophy. Int J Immunopathol Pharmacol 2007; 20(3): 519-27.

[132] Schneider A, Haas S, Hildenbrand R, *et al*. Enhanced expression of interleukin-18 in serum and pancreas of patients with chronic pancreatitis. World J Gastroenterol 2006; 12(40): 6507-14.

[133] Sennello J, Fayad R, Pini M, *et al*. Interleukin-18, together with interleukin-12, induces severe acute pancreatitis in obese but not in nonobese leptin-deficient mice. PNAS 2008; 105(23): 8085-90.

[134] Oikawa Y, Shimada A, Kasuga A, *et al*. Systemic administration of IL-18 promotes diabetes development in young nonobese diabetic mice. J Immunol 2003; 171(11): 5865-75.

[135] Nicoletti F, Di Marco R, Papaccio G, *et al*. Essential pathogenic role of endogenous IL-18 in murine diabetes induced by multiple low doses of streptozotocin. Prevention of hyperglycemia and insulitis by a recombinant IL-18-binding protein: Fc construct. Europ J Immunol 2003; 33(8): 2278-86.

[136] Gerdes N, Sukhova GK, Libby P, Reynolds RS, Young JL, Schonbeck U. Expression of interleukin (IL)-18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells, and macrophages: Implications for artherogenesis. J Exp Med 2002; 195(2): 245-57.

[137] Yoshida A, Kohka Takahashi H, Nishibori M, *et al*. IL-18-induced expression of intercellular adhesion molecule-1 in human monocytes: Involvement in IL-12 and IFN-[gamma] production in PBMC. Cell Immunol 2001; 210(2): 106-15.

[138] Chandrasekar B, Mummidi S, Valente AJ, *et al*. The pro-atherogenic cytokine interleukin-18 induces CXCL16 expression in rat aortic smooth muscle cells *via* MyD88, interleukin-1 receptor-associated kinase, tumor necrosis factor receptor-associated factor 6, c-Src, phosphatidylinositol 3-kinase, Akt, c-Jun N-terminal kinase, and activator protein-1 signaling. J Biol Chem 2005; 280(28): 26263-77.

[139] Mallat Z, Corbaz A, Scoazec A, *et al*. Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. Circulation 2001; 104(14): 1598-603.

[140] Aso Y, Okumura K-i, Takebayashi K, Wakabayashi S, Inukai T. Relationships of plasma interleukin-18 concentrations to hyperhomocysteinemia and carotid intimal-media wall thickness in patients with type 2 diabetes. Diabetes Care 2003; 26(9): 2622-7.

[141] Yamagami H, Kitagawa K, Hoshi T, *et al*. Associations of serum IL-18 levels with carotid intima-media thickness. Arterioscler Thromb Vasc Biol 2005; 25(7): 1458-62.

[142] Yamaoka-Tojo M, Tojo T, Masuda T, *et al*. C-reactive protein-induced production of interleukin-18 in human endothelial cells: a mechanism of orchestrating cytokine cascade in acute coronary syndrome. Heart Vessels 2003; 18(4): 183-7.

[143] Yamashita H, Shimada K, Seki E, Mokuno H, Daida H. Concentrations of interleukins, interferon, and C-reactive protein in stable and unstable angina pectoris. Am J Cardiol 2003; 91(2): 133-6.

[144] Mallat Z, Corbaz A, Scoazec A, *et al*. Interleukin-18/Interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability. Circ Res 2001; 89(7): e41-5.

[145] Blankenberg S, Tiret L, Bickel C, *et al*. Interleukin-18 is a strong predictor of cardiovascular death in stable and unstable angina. Circulation 2002; 106(1): 24-30.

[146] He G, Andersen S, Haugaard, *et al*. Plasminogen activator inhibitor type 1 (PAI-1) in plasma and adipose tissue in HIV-associated lipodystrophy syndrome. Implications of adipokines. Eur J Clin Invest 2005; 35(9): 583-90.

[147] Wang M, Markel T, Meldrum D. Interleukin 18 in the heart. Shock 2008; 30(1): 3-10.

[148] Chandrasekar B, Valente A, Freeman G, Mahimainathan L, Mummidi S. Interleukin-18 induces human cardiac endothelial cell death *via* a novel signaling pathway involving NF-kappaB-dependent PTEN activation. Biochem Biophys Res Commun 2006; 339(3): 956-63.

[149] Chandrasekar B, Mummidi S, Claycomb WC, Mestril R, Nemer M. Interleukin-18 is a pro-hypertrophic cytokine that acts through a phosphatidylinositol 3-kinase-phosphoinositide-dependent kinase-1-Akt-GATA4 signaling pathway in cardiomyocytes. J Biol Chem 2005; 280(6): 4553-67.

[150] de Ferranti S, Mozaffarian D. The perfect storm: Obesity, adipocyte dysfunction, and metabolic consequences. Clin Chem 2008; 54(6): 945-55.

[151] Stein J. Managing cardiovascular risk in patients with HIV infection. J Acquir Immune Defic Syndr 2005; 38(2): 115-23.

[152] Mangili A, Gerrior J, Tang AM, *et al*. Risk of cardiovascular disease in a cohort of HIV-infected adults: A study using carotid intima-media thickness and coronary artery calcium score. Clin Infect Dis 2006; 43(11): 1482-9.

[153] McComsey G, O'Riordan M, Hazen S, *et al*. Increased carotid intima media thickness and cardiac biomarkers in HIV infected children. AIDS 2007; 21(8): 921-7.

[154] Tabib A, Greenland T, Mercier I, Loire R, Mornex J. Coronary lesions in young HIV-positive subjects at necropsy. Lancet 1992; 340(8821): 730.

[155] Constans J, Marchand J, Conri C, *et al*. Asymptomatic atherosclerosis in HIV-positive patients: A case-control ultrasound study. Ann Med. 1995; 27(6): 683-5.

[156] Ances BM, Ellis RJ. Dementia and neurocognitive disorders due to HIV-1 infection. Semin Neurol 2007; 27(01): 086-92.

[157] Aquaro S, Svicher V, Ronga L, Perno C, Pollicita M. HIV-1-associated dementia during HAART therapy. Recent Pat CNS Drug Discov 2008; 3(1): 23-33.

[158] Brabers N, Nottet H. Role of the pro-inflammatory cytokines TNF-alpha; and IL-1beta; in HIV-associated dementia. Eur J Clin Invest 2006; 36(7): 447-58.

[159] von Giesen H, Jander S, Köller H, Arendt G. Serum and cerebrospinal fluid levels of interleukin-18 in human immunodeficiency virus type 1-associated central nervous system disease. J Neurovirol 2004; 10(6): 383-6.

[160] Bossù P, Ciaramella A, Salani F, *et al*. Interleukin-18 produced by peripheral blood cells is increased in Alzheimer's disease and correlates with cognitive impairment. Brain Behav Immun 2008; 22(4): 487-92.

[161] Kalina U, Ballas K, Koyama N, *et al*. Genomic organization and regulation of the human interleukin-18 gene. Scand J Immunol 2000; 52(6): 525-30.

[162] Sugama S, Conti B. Interleukin-18 and stress. Brain Res Rev 2008; 58(1): 85-95.

[163] Thompson SR, Humphries SE. Interleukin-18 genetics and inflammatory disease susceptibility. Genes Immun 2007; 8(2): 91-9.

[164] Giedraitis V, He B, Huang W-X, Hillert J. Cloning and mutation analysis of the human IL-18 promoter: a possible role of polymorphisms in expression regulation. Journal of Neuroimmunology 2001; 112(1-2): 146-52.

[165] Segat L, Bevilacqua D, Boniotto M, *et al*. IL-18 gene promoter polymorphism is involved in HIV-1 infection in a Brazilian pediatric population. Immunogenetics 2006; 58(5): 471-3.

[166] Netea MG, Joosten LAB, Lewis E, *et al*. Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. Nat Med 2006; 12(6): 650-6.

[167] Naugler WE, Karin M. The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer. Trends Mol Med 2008; 14(3): 109-19.

[168] Zilverschoon G, Tack C, Joosten L, Kullberg B, van der Meer J, Netea M. Interleukin-18 resistance in patients with obesity and type 2 diabetes mellitus. Int J Obes 2008; 32(9): 1407-14.

[169] Aste-Amezaga M, D'Andrea A, Kubin M, Trinchier iG. Cooperation of natural killer cell stimulatory factor/interleukin-12 with other stimuli in the induction of cytokines and cytotoxic cell-associated molecules in human T and NK cells. Cell Immunol 1994; 156(2): 480-92.

[170] Fehniger T, Shah M, Turner M, *et al*. Differential cytokine and chemokine gene expression by human NK cells following activation with IL-18 or IL-15 in combination with IL-12: implications for the innate immune response. J Immunol 1999; 162(8): 4511-20.

[171] Walker W, Aste-Amezaga M, Kastelein RA, Trinchieri G, Hunter CA. IL-18 and CD28 use distinct molecular mechanisms to enhance NK cell production of IL-12-induced IFN-{gamma}. J Immunol 1999; 162(10): 5894-901.

[172] Agaugue S, Marcenaro E, Ferranti B, Moretta L, Moretta A. Human natural killer cells exposed to IL-2, IL-12, IL-18, or IL-4 differently modulate priming of naive T cells by monocyte-derived dendritic cells. Blood 2008; 112(5): 1776-83.

[173] Yamanaka R, Honma J, Tsuchiya N, Yajima N, Kobayashi T, Tanaka R. Tumor lysate and IL-18 loaded dendritic cells elicits Th1 response, tumor-specific CD8+ cytotoxic T cells in patients with malignant glioma. J Neurooncol 2005; 72(2): 107-13.

[174] Wild JS, Sigounas A, Sur N, *et al*. IFN-{gamma}-inducing factor (IL-18) increases allergic sensitization, serum IgE, Th2 cytokines, and airway eosinophilia in a mouse model of allergic asthma. J Immunol 2000; 164(5): 2701-10.

[175] Yoshimoto T, Mizutani H, Tsutsui H, *et al*. IL-18 induction of IgE: dependence on CD4+ T cells, IL-4 and STAT6. Nat Immunol 2000; 1(2): 132-7.

[176] Yoshimoto T, Tsutsui H, Tominaga K, *et al*. IL-18, although antiallergic when administered with IL-12, stimulates IL-4 and histamine release by basophils. PNAS 1999; 96(24): 13962-6.

[177] Hashimoto W, Osaki T, Okamura H, *et al*. Differential antitumor effects of administration of recombinant IL-18 or recombinant IL-12 are mediated primarily by Fas-Fas ligand- and perforin-induced tumor apoptosis, respectively. J Immunol 1999; 163(2): 583-9.

[178] Pintaric M, Gerner W, Saalmüller A. Synergistic effects of IL-2, IL-12 and IL-18 on cytolytic activity, perforin expression and IFN-gamma production of porcine natural killer cells. Vet Immunol Immunopathol 2008; 121(1-2): 68-82.

[179] Yang Y, Li X, Hong J, *et al*. Interleukin-18 enhances glucose uptake in 3T3-L1 adipocytes. Endocrine 2007; 32(3): 297-302.

[180] Ye XJ, Tang B, Ma Z, *et al*. The effects of interleukin-18 on rat articular chondrocytes: a study of mRNA expression and protein synthesis of proinflammatory substances. Clin Exp Immunol 2007; 149(3): 553-60.

[181] Amin M, Mansfield P, Pakozdi A, *et al*. Interleukin-18 induces angiogenic factors in rheumatoid arthritis synovial tissue fibroblasts *via* distinct signaling pathways. Arthritis Rheum 2007; 56(6): 1787-97.

[182] Kim J, Shao Y, Kim SY, *et al*. Hypoxia-induced IL-18 increases hypoxia-inducible factor-1{alpha} expression through a Rac1-dependent NF-{kappa}B pathway. Mol Biol Cell 2008; 19(2): 433-44.

[183] Inoue H, Hiraoka K, Hoshino T, *et al*. High levels of serum IL-18 promote cartilage loss through suppression of aggrecan synthesis. Bone 2008 06/01; 42(6): 1102-10.

[184] Olee T, Hashimoto S, Quach J, Lotz M. IL-18 is produced by articular chondrocytes and induces proinflammatory and catabolic responses. J Immunol 1999; 162(2): 1096-100.

[185] Yamada N, Niwa S, Tsujimura T, *et al*. Interleukin-18 and interleukin-12 synergistically inhibit osteoclastic bone-resorbing activity. Bone 2002; 30(6): 901-8.

[186] Favre D, Lederer S, Kanwar B, *et al*. Critical loss of the balance between Th17 and T regulatory cell populations in pathogenic SIV infection. PLoS Pathog 2009; 5(2): e1000295.

[187] Ozturk C, OOzge A, Yalın O, *et al*. The diagnostic role of serum inflammatory and soluble proteins on dementia subtypes: Correlation with cognitive and functional decline. Behav Neurol 2007; 18(4): 207-15.


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# Article 3

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# Role of Interleukin-18 in the Development and Pathogenesis of AIDS

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## Abstract

***Interleukin-18 is a proinflammatory, proapoptotic, and proatherogenic cytokine belonging to the interleukin-1 family of cytokines. The cytokine exerts many unique immunologic and biological effects. It is produced as a biologically inactive and leaderless precursor protein, which must be cleaved into its mature form by caspase-1. The caspase-1 also exists in an inactive precursor in the cytosol and needs proteolytic auto-cleavage, which is catalyzed by the assembly of a multi-protein complex called inflammasome.***

***Inside the circulation, interleukin-18 is bound to its naturally occurring antagonist called interleukin-18 binding protein. The antagonist is induced as a negative feedback to increased interleukin-18 production. It protects body cells and tissues from the potentially destructive and harmful proinflammatory effects of the cytokine.***

***Several researchers have reported that the concentrations and biological activities of the cytokine are increased in the circulation of HIV-infected patients. Unlike interleukin-18, the concentrations of its antagonist, interleukin-18 binding protein, are decreased in these persons. The cytokine may play a major role in the development and pathogenesis of AIDS in HIV-infected persons. Insufficient/lack of interleukin-12 and related cytokines may compromise the ability of interleukin-18 to induce interferon-gamma production from natural killer and T-cells. By inducing production of T-helper 2-type cytokines like interleukin-4, -5, -9, and -13 from basophils and mast cells, interleukin-18 promotes the development and differentiation of CD4$^{+}$ naive T-cells into T-helper 2-type effector cells, which blunt anti-HIV immunity. The effect may be more pronounced in HIV-infected persons with compromised production of interleukin-12. Interleukin-18 also directly enhances viral replication. Because of its proapoptotic effects, the cytokine decreases survivability and promotes the death of various immune and nonimmune cells. It has also been documented to play a role in the depletion and wasting of subcutaneous fat from the limbs and face. The wasting is a characteristic feature of HIV-associated lipodystrophy. The cytokine is also likely to be involved in the higher incidence of atherosclerotic plaques and systemic insulin resistance in these patients.***

***Finally, increased production of the cytokine in the brain may lead to motor and cognitive dysfunctions, leading to the development of HIV-associated dementia.***

***In conclusion, increased interleukin-18 concentrations in HIV-infected persons are likely to play an important role in the development and progression of the infection toward AIDS and associated clinical***

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***conditions. Therefore, its neutralization may represent an appropriate and useful immunotherapeutic strategy in these patients. It may delay AIDS progression and improve the immune status of infected persons. The best way to achieve this goal may be using exogenous interleukin-18 binding protein.***
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## Key words

***AIDS. Cytokines. HIV-1. IL-18. IL-18BP. Lipodystrophy. HIV-associated dementia.***

## Introduction

Several studies have shown that interleukin-18 (IL-18) concentrations are increased in the circulation of HIV-infected persons as compared to HIV-seronegative healthy subjects. Here we provide an overview of our current state of knowledge on the biology of this cytokine and discuss, in the light of published literature, how these increased concentrations of the cytokine might contribute towards the pathogenesis of AIDS and its associated clinical conditions like lipodystrophy, systemic insulin resistance, and dementia.

## A unique proinflammatory cytokine

Interleukin-18 represents the fourth member of the IL-1 family of cytokines. It is a multifunctional and pleiotropic, proinflammatory cytokine with many unique biological effects (Table 1). The cytokine is produced by a wide variety of cells including macrophages, dendritic cells, keratinocytes, adipocytes, Kupffer cells, intestinal and respiratory tract epithelial cells, microglial cells, ependymal cells, and certain neurons in the brain (reviewed[1-3]).

Interleukin-18 gene expression has been documented in several organs and tissues of the body, e.g. liver, lungs, thymus, heart, placenta, kidneys, pancreas, adipose tissue, brain, etc. Physical and emotional stress has been shown to induce IL-18 production from adrenal cortex[4,5]. Like IL-1β, the prototype member of the family, IL-18 secretion does not follow the classical secretory pathway via endoplasmic reticulum and Golgi apparatus. Instead, the cytokine is produced as a leaderless and biologically inactive 24 kDa precursor protein called Pro-IL-18, which is cleaved by IL-1β converting enzyme (ICE; more commonly known as caspase-1) to produce 18 kDa mature and biologically active cytokine[6,7].

It is noteworthy that caspase-1 itself exists in an inactive 45 kDa precursor form whose activation requires assembly of multi-unit complexes involving certain nucleotide-binding and oligomerization domain (NOD)-like proteins. These complexes, known as inflammasomes, recruit and activate caspase-1 precursor molecules (reviewed[8,9]). The process leads to IL-18 maturity and secretion.

As shown in figure 1, an increased production of biologically active IL-18 requires two distinct stimuli: one increases IL-18 gene expression at mRNA and protein levels and usually comes from recognition of pathogen products by a pattern-recognizing receptor (e.g. toll-like receptor); the second signal causes inflammasome assembly, caspase-1 activation, and secretion of mature IL-18.

Understanding the stimuli and the molecular mechanisms that lead to inflammasome assembly and the secretion of biologically active IL-18 is a burgeoning area of research. A variety of cellular products (e.g. extracellular adenosine triphosphate, amyloid β crystals,

**Table 1. Some unique properties of interleukin-18**

- Despite being a member of the IL-1 family, IL-18 has several unique properties:
- It does not induce fever; but does induce sleep and anorexia
- In synergy with IL-12, it induces IFNγ production from natural killer and T-cells
- It induces IL-4, IL-5, IL-9, IL-13 and histamine from mast cells and basophils
- Its effects are proapoptotic on target cells, while other members of the family exert pro-survival effects
- It is also produced in adrenal cortex in response to physical and emotional stress
- Its antitumor effects are uniquely mediated by enhanced FasL-mediated cytotoxicity of natural killer cells and T-cells
- Implicated in acute hepatic injury in endotoxemia and mouse models of viral hepatitis
- One of the rare cytokines that are kept inactive in the body by a naturally produced antagonist
- It promotes the development of atherosclerotic plaques
- It is involved in the maintenance of homeostasis of energy: its enhanced concentrations promote insulin resistance

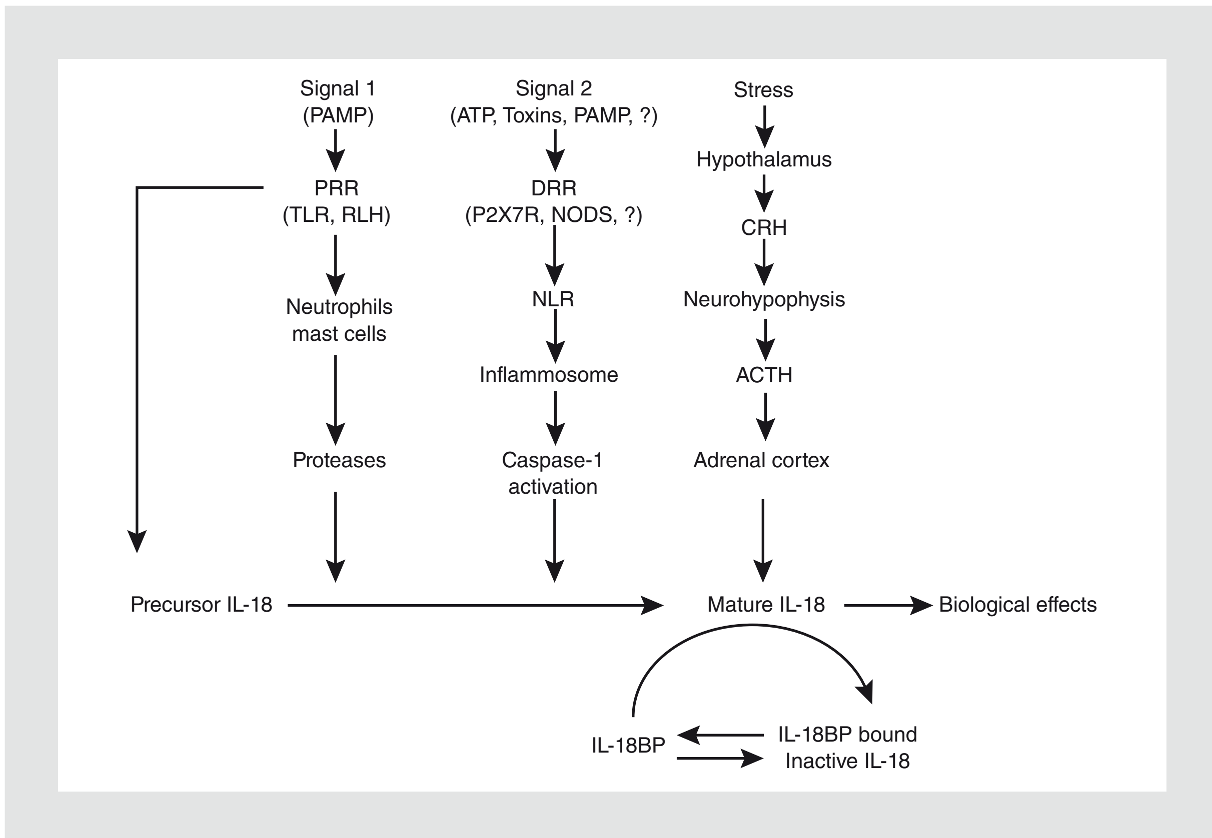


**Figure 1.** *Production, processing and control of interleukin-18. The figure depicts two signaling pathways needed to produce precursor IL-18. In addition to pathogens and their products, stress can also induce IL-18 production in adrenal gland and in certain brain cells (not shown). IL: interleukin; BP: binding protein; ACTH: adrenal corticotrophic hormone; CRH: corticotrophic releasing hormone; DRR: danger recognizing receptors; NOD: nucleotide oligomerization and dimerization domain-containing proteins; NLR: NOD-like receptors; PAMP: pathogen-associated molecular patterns; PRR: pattern recognizing receptors; RLH: RIG-like helicase. A question mark (?) indicates unknown receptors or stimuli.*

reactive oxygen, and nitrogen species), pathogen products (e.g. bacterial and viral nucleic acids, bacterial muramyl dipeptide, bacterial toxins) and environmental pollutants (e.g. silica particles, aluminum compounds) are known to cause inflammasome assembly[10-14]. Along with the mature form, the unprocessed precursor form is also released from IL-18-producing cells. Certain proteases e.g. matrix metalloprotease (MMP)-9, neutrophil-derived proteinase-3 and elastase, mast cell-derived chymase, etc. can process the extracellular IL-18 precursor into biologically active forms[1-3].

Once secreted, mature IL-18 is subjected to a further layer of control; a protein (named as IL-18 binding protein; IL-18BP) specifically binds and inactivates mature IL-18 in the circulation. The IL-18BP-bound cytokine is able to bind the "α" chain of IL-18 receptor (IL-18R; see below), but is unable to recruit its "β" chain. Consequently, it cannot transduce signals in target cells[15,16].

The human IL-18BP exists in four isoforms, which result from alternate splicing of the IL-18BP mRNA[15]. The isoforms have been named "a-d"; only "a" and "c" can bind and inactivate IL-18. It is noteworthy that the "a" isoform exhibits a tenfold higher affinity for IL-18 as compared with the "c" isoform. The former isoform accounts for practically all the IL-18 neutralizing activity in the human body. It binds IL-18 with high affinity and low dissociation rate in 1:1 stoichiometric complexes[17].

The IL-18BP is produced constitutively from several types of cells and tissues in the body. The protein is normally present in the circulation in 20-fold molar excess as compared to IL-18[17]. This ensures protection from tissue damage, which otherwise might occur from uncontrolled biological activities of the cytokine.

The production of IL-18BP is also enhanced as a negative feedback mechanism in response to enhanced IL-18 production. For example, increased levels of IL-18 are accompanied by increased levels of IL-18BP in the circulation of mice during sepsis[17]. *In vitro* studies have shown that interferon gamma (IFNγ) acts as a powerful stimulus for inducing expression of

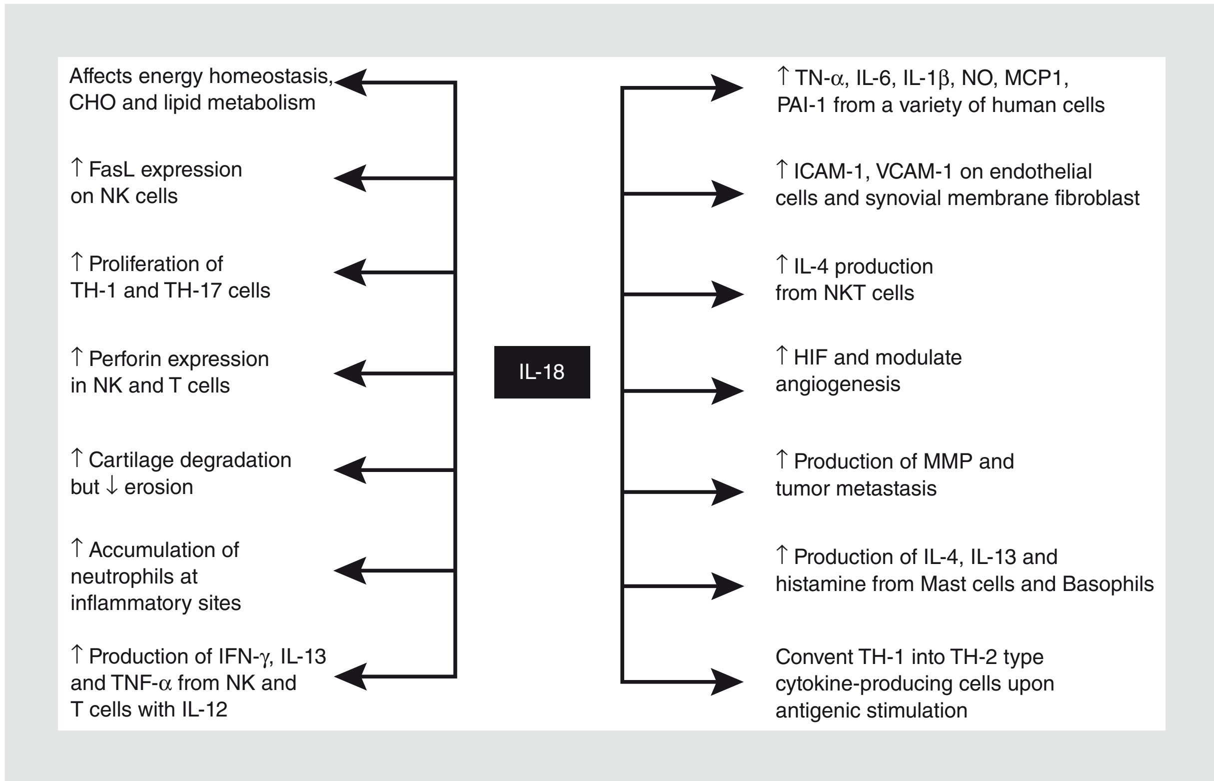


**Figure 2.** *Biological effects of interleukin-18. IL: interleukin; TH: T helper; NK: natural killer; AT: adipose tissue; CHO: carbohydrate; ICAM-1: intercellular cell adhesion molecule 1; MCP-1: macrophage chemotactic protein 1; MMP: matrix metalloproteinase; NO: nitrous oxide; PAI-1: plasminogen activator inhibitor 1; VCAM: vascular cell adhesion molecule; VEC: vascular endothelial cells.*

IL-18BP. Production of IL-18BP mediated by this interferon is an important factor in the operation of this negative feedback mechanism[18,19].

Interestingly, several poxviruses carry a homolog of the IL-18BP gene in their genomes. The viral IL-18BP acts as a virulence factor and antagonizes the virus-induced IL-18 responses of the infected host[20,21]. The IL-18BP acts as an anti-inflammatory and immunosuppressive mediator[2,3,22], and it represents an ideal therapeutic tool for neutralizing IL-18 in chronic inflammatory diseases.

Interleukin-18 exerts its biological effects on target cells and tissues via IL-18 receptors (IL-18R; Fig. 2). The IL-18R is heterodimeric and consists of two distinct polypeptide chains: α and β. Immunoglobulin (Ig)-like domains are present in the extracellular regions of the receptor chains and bind IL-18. Toll-IL-1 receptor (TIR) domains present in the cytoplasmic tails of the receptor chains transduce signals in the target cells[2,3].

The cytokine initiates inflammatory responses by inducing production of several proinflammatory cytokines and chemokines (e.g. TNFα, IL-8, IL-1β, MIP-1α, NO, MMP) from a variety of human cells. It induces accumulation of neutrophils in the lungs and liver during septicemia[23]. The cytokine chemo-attracts dendritic cells to the site of viral infection and causes their maturation[24]. It also plays an important role in enhancing adhesion of dendritic cells, macrophages, natural killer (NK) cells, and T-cells to vascular endothelial cells by enhancing expression of vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1)[25].

The most significant biological effect of IL-18 is induction of IFNγ production from NK, T-, and NK T-cells. However, it does so in concert with other cytokines, e.g. IL-12, IL-15, or IL-21. Interleukin-18 and IL-12 together represent the most powerful stimulus in inducing IFNγ from NK and T-cells[26] (reviewed[27]). In fact, the cytokine was originally discovered in 1989 as an "IFNγ-inducing factor" that was present in the serum of the Bacillus Calmette-Guerin-infected mice after challenge with lipopolysaccharide. The "factor" induced IFNγ production from IL-2-activated mouse splenocytes[23]. Several years later it was named as IL-18 upon cloning and expression of its gene[28,29]. Many of the cytokine's biological effects can be attributed to its ability to induce IFNγ. The interferon induces expression of IL-12Rβ1 chain on the surface of naive $CD4^+$ T-cells and renders them responsive to IL-12, which induces their differentiation into T-helper 1 (Th1) effector T-cells. The interferon also

inhibits differentiation of these naive cells into Th2- or Th17-type effector cells[30,31]. During the differentiation process, IL-12 induces IL-18Rβ chain on $CD4^+$ T-cells. Consequently, IL-18 can amplify the Th1-type $CD4^+$ T-cell responses. These responses have been shown to be more effective in controlling intracellular pathogens like HIV-1.

It is noteworthy that in the absence of collaborating cytokines (e.g. IL-12), IL-18 has little ability to induce IFNγ from NK and T-cells. However, it can also exert many biological effects that are independent of its IFNγ-inducing ability. The cytokine induces IL-4, IL-5, IL-9, and IL-13 from basophils, mast cells, and ligand-activated NK T-cells[32]. Furthermore, the presence of IL-18 can efficiently convert $CD4^+$ effector T-cells into Th2-type cytokine-producing cells upon their antigen-specific activation[33,34]. The Th2-type cytokines promote allergic inflammatory responses (mastocytosis, eosinophilia, and IgE production) needed to expel extracellular parasites. Thus, an excess of IL-18 production would blunt Th1-type responses and promote Th2-type responses in the body. Interleukin-18 augments the FasL-mediated cytotoxicity of NK and T-cells[35,36]. The cytokine exerts its antitumor effects mainly via the Fas/FasL pathway. Few antitumor effects of the cytokine were observed in Fas or FasL knockout (KO) mice. The IL-18-mediated enhanced FasL expression has been implicated in the cytokine-induced hepatotoxicity in septicemia and mouse models of hepatitis[37].

## Imbalanced production of interleukin-18 and interleukin-18 binding protein in HIV infection

Several researchers have consistently reported increased concentrations of IL-18 in the circulation of HIV-infected persons[38,39] (reviewed[40]). The cytokine concentrations did not correlate with viral load or with $CD4^+$ T-cell counts in these patients[38,39]. More recent studies have shown that the cytokine increases in the circulation of infected persons early in the course of the infection, and its concentrations correlate with viral loads in untreated persons[41,42]. The cytokine levels decrease in HAART-treated patients, but they remain above physiological concentrations. The authors suggested using the levels of the cytokine as a measure of response to therapy[43]. Another study demonstrated that seroconversion in HIV-infected persons was accompanied by increased levels of the cytokine in the circulation, and the levels correlated with viral loads in these patients[44].

As stated above, IL-18 production is accompanied by a negative feedback mechanism, which induces production of its antagonist IL-18BP. We have found that, in sharp contrast to IL-18, the concentrations of IL-18BP are significantly decreased in the circulation of HIV-infected persons as compared to HIV-seronegative healthy subjects (Iannello A, et al. submitted). Thus, IL-18-induced negative-feedback mechanisms appear to have become defective in HIV-infected persons. This results in increased concentrations of "free" and biologically active IL-18 in the circulation of HIV-infected persons. Indeed, we have previously shown that IL-18 present in the sera of HIV-infected persons is biologically active[38]. As mentioned earlier, IFNγ plays an important role in inducing IL-18BP from human cells, and IL-18 needs to act in concert with other cytokines, like IL-12 or IL-15, for inducing production of this interferon from human cells. Given that the production of IL-12 and IL-15 decreases in HIV-infected persons[45-47] (reviewed[48]), this may result in a decreased ability of IL-18 to induce IFNγ from NK and T-cells. A decreased production of IFNγ may be at least in part responsible for a decreased production of IL-18BP in HIV-infected persons.

## Implications of increased interleukin-18 levels for HIV infection

An increased production of IL-18 as well as an increase in its biological activities may contribute to the development of AIDS (Fig. 3) as described below.

### *Adverse effects on antiviral immunity*

Because of its ability to induce IFNγ from NK and T-cells, IL-18 may play a role in protecting the host from intracellular pathogens. As mentioned earlier, IL-18 could promote type 1 or type 2 immune responses, depending upon the context. Induction of IFNγ and promotion of type 1 immune responses by IL-18 depends upon the presence of collaborating cytokines like IL-12, IL-15, or IL-21. In the absence/insufficiency of these cytokines, IL-18 has little ability to induce IFNγ from NK and T-cells. Under these conditions, IL-18 may in fact blunt these responses by inducing IL-4, IL-5, IL-9, IL-13, and histamine from basophils and mast cells. These cytokines promote the development and differentiation of Th2-type $CD4^+$ T-cells, which induce type 2 immune responses. A decreased production of IFNγ and a predominance of type 2 immune responses frequently occur in HIV-infected persons and have been implicated in the development of AIDS[49-51].

Increased concentrations of IL-18 have been documented in several chronic inflammatory conditions. Levels of IL-18 in these conditions were shown to correlate with reduced NK cell numbers[52,53]. We have found that

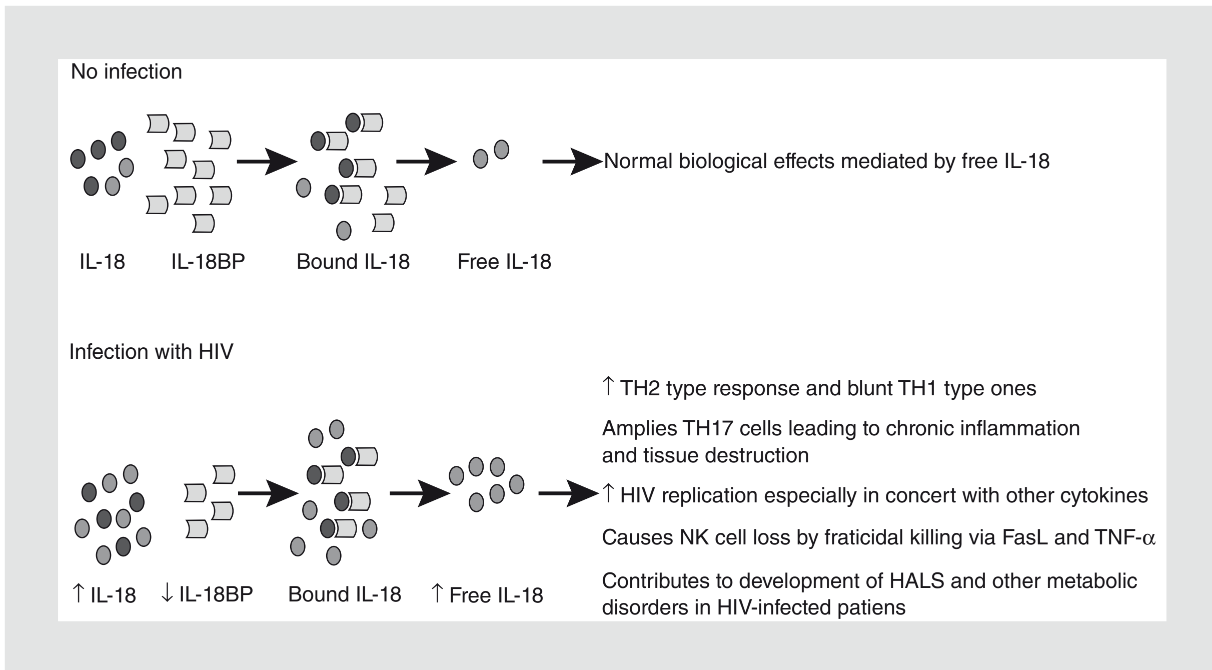


**Figure 3.** *Potential contributions of interleukin-18 to AIDS pathogenesis. The figure depicts various mechanisms by which IL-18 might contribute towards AIDS pathogenesis. Note increase in free IL-18 concentrations in the circulation of HIV-infected persons. IL: interleukin; TH: T helper; NK: natural killer; BP: binding protein; HALS: HIV-associated lipodystrophy syndrome.*

a similar situation exists in HIV-infected persons[54]. We further found that the cytokine induces FasL expression on primary human NK cells, and decreases their survivability by reducing the expression of an antiapoptotic protein, Bcl-XL. These changes result in fratricidal killing of NK cells in their *in vitro* cultures. It is conceivable that a chronic exposure of NK cells to high concentrations of IL-18 *in vivo* may cause their depletion and, hence, defective innate immunity. The cytokine has been shown to enhance FasL-mediated cytotoxicity of murine $CD4^+$ Th1 cells[35]. Although not formally shown, it is also likely to enhance expression of FasL in human $CD4^+$ Th1 cells. However, depletion of this type of cell in chronic inflammatory conditions has not been reported. It is likely that the cytokine exerts differential effects on the two types of target cells and does not decrease the survivability of $CD4^+$ Th1 cells. By inducing enhanced expression of FasL on NK and $CD4^+$ Th1 cells, the cytokine may promote the destruction of normal Fas-positive cells in the body and promote autoimmunity. It is noteworthy that increased Fas/FasL-mediated killing has been implicated in the immunopathogenesis of AIDS (reviewed[55]).

## *Effects on HIV replication*

Proinflammatory, immune-enhancing cytokines often enhance HIV replication. Concerning the effects of IL-18 on HIV replication, controversial results have been reported. The cytokine has been shown to enhance the viral replication in acutely or chronically infected human monocytic and T-cell lines[56-58]. However, it was reported to inhibit HIV replication in human peripheral blood mononuclear cells (PBMC)[59]. The inhibition was ascribed, at least in part, to IL-18-mediated IFNγ production in the human cell cultures. However, it is important to note that production of this interferon from PBMC in response to IL-18 becomes compromised in HIV-infected persons[41]. This compromise could be due to increased circulating concentrations of immunosuppressive cytokines, like IL-10, IL-32, and transforming growth factor-beta (TGFβ), and/or due to decreased concentrations of immune-enhancing cytokines like IL-12, IL-15, and IL-21[45-47,60-62] (reviewed[48]). In this regard, we have found that IL-18 enhances HIV replication in *in vitro* infected purified human $CD4^+$ T-cells, but has no effect on viral replication in *in vitro* generated human monocyte-derived macrophages (Iannello A, et al. submitted). Much of the controversy concerning the effects of IL-18 on HIV replication probably could be ascribed to differences in the expression of IL-18R and/or concomitant induction of IL-18BP from human cells. In IL-18R-positive cells, the cytokine is more likely to induce HIV replication. An enhancing effect of IL-18 on HIV replication is supported by results from *in vivo* studies in animal models. In these

studies, pathogenic, but not nonpathogenic, chimeric SIV/HIV-1 (SHIV) viruses caused increased IL-18 levels. The infected animals with increased levels of the cytokine experienced a more rapid loss of $CD4^+$ T-cells and higher set points for viral load[63]. The cytokine seemed to promote disease progression in these studies, although it would have been more desirable to investigate the effects of *in vivo* neutralization and of administration of the cytokine on viral replication and disease progression. In NOD-SCID mice grafted with human cells, IL-18 also enhanced HIV replication[64]. Taken together, these studies strongly suggest that IL-18 promotes HIV replication in HIV-infected persons.

## Biological effects of interleukin-18 beyond immune system

The IL-18Rs are expressed on the surface of adipocytes, vascular endothelial cells, and in certain brain cells. This allows IL-18 to exert biological effects in the body beyond immune system. Like other proinflammatory cytokines, it inhibits lipogenesis and promotes lipolysis (reviewed[65,66]). The transcripts for IL-18 and its receptor have been detected in adipose tissue including adipocytes[67]. Consequently, the cytokine may cause lipo-depletion and it is not surprising that the cytokine has been implicated in HIV-associated lipodystrophy syndrome. The syndrome, first described in 1998[68], is characterized by a redistribution of body fat. The patients usually lose subcutaneous fat on face ("empty cheek syndrome"), arms, legs, and trunk, and gain fat on the dorsocervical region ("buffalo hump condition"), breast, and viscera (reviewed[66]). HIV-associated lipodystrophy syndrome also occurred in HIV-infected persons before the advent of antiretroviral therapies; however, the use of HAART has significantly increased its incidence and severity. Both reverse transcriptase inhibitors and protease inhibitors have been implicated in hastening this syndrome[66]. About one-third of HIV-infected persons undergoing antiretroviral therapy experience it in one form or another.

Experimental studies have demonstrated that inhibition of lipogenesis (differentiation of pre-adipocytes into adipocytes) and increased lipolysis in adipocytes are responsible for loss of fat in HIV-infected persons[66,69]. Increased numbers of adipocytes undergoing apoptosis in the adipose tissues have been observed in these patients[70]. It is believed that one of the mechanisms that underlie the causation of this syndrome is induction of proinflammatory cytokines, which may result from direct effects of the virus, viral gene products, and/or antiviral drugs. Among these cytokines, the potential role of IL-18 in the etiopathogenesis of this syndrome is beginning to emerge. Although the levels of the cytokine increase in the circulation of HIV-infected persons, they are higher in the patients with HIV-associated lipodystrophy syndrome than in the patients without it[71]. The gene is also expressed at higher levels in the adipose tissues of these patients. In two separate studies involving subcutaneous fat of the limbs and femoral gluteal tissues, the level of IL-18 gene expression correlated with the degree of lipoatrophy[71,72]. Another hallmark of HIV-associated lipodystrophy syndrome is diminution in the circulating levels of adiponectin, an adipocyte-produced anti-inflammatory mediator, which increases insulin sensitivity. It has been demonstrated *in vitro* that IL-18 inhibits production of adiponectin in the adipose tissues[73]. Increased concentration of IL-18 in the circulation of HIV-infected persons may be at least partially responsible for declining adiponectin levels in HIV-associated lipodystrophy syndrome as well as in obesity and type 2 diabetes.

Interleukin-18 has been associated with obesity. The condition is accompanied by low-grade inflammation resulting from production of several proinflammatory cytokines including IL-18 from adipose tissues. Increased concentrations of the cytokine have been reported in obese persons[65,74-76]. The cytokine, like other proinflammatory cytokines such as tumor necrosis factor alpha (TNF$\alpha$) and IL-15, inhibits lipogenesis and promotes lipolysis. This may explain phenotype of the IL-18 or IL-18R KO mice, which become hyperphagic, obese, and develop insulin resistance[77]. Intracerebroventricular, but not intraperitoneal, injections of exogenous IL-18 reverse these effects.

Interestingly, continuous feeding of the cytokine in mice also leads to insulin resistance and the development of type 2 diabetes[78]. The cytokine appears to have differential effects on food intake and lipid metabolism when administered peripherally (intraperitoneally or intravenous) or centrally (intracerebroventricularly).

It is noteworthy that several manifestations of disturbances in homeostasis of energy, lipid, and carbohydrate metabolism, e.g. hypercholesterolemia, dyslipidemia (decreased HDL, hypertriglyceridemia, increased LDL and VLDL), increased fasting levels of glucose and insulin in the blood, etc. had occurred in HIV-infected persons without receiving antiretroviral therapy. The situation would have been exacerbated with the advent of HAART had these patients not been treated with lipolytic agents[78-81]. It has been shown that serum concentrations of IL-18 are significantly higher in HIV-infected patients with hypertriglyceridemia as compared to patients having normal triglyceride levels[82]. In view of the effects of this

cytokine on lipid metabolism and energy homeostasis, it is much more likely to be causally involved in precipitating these manifestations in these patients.

It is noteworthy that systemic insulin resistance is a major complication occurring in HIV-infected patients, especially in those presenting HIV-associated lipodystrophy syndrome[66,83]. Several studies have shown that IL-18 may contribute towards insulin resistance in these patients as well as in type 2 diabetes patients[74,75,78,84,85]. As currently understood, the insulin resistance develops gradually as a result of obesity-associated, chronic low-grade inflammation (reviewed[75,84,86]). The levels of the cytokine correlate with those of glucose and triglycerides in obese and type 2 diabetes patients[76]. Furthermore, elevated levels of the cytokine, especially with IL-6 or C-reactive protein, predict impending type 2 diabetes in middle-aged men and women[85]. The concentrations of several proinflammatory cytokines, e.g. IL-6, TNFα, C-reactive protein, IL-18, etc. are often increased in the circulation of obese persons. Interestingly, increasing IL-18 concentrations in the circulation predict the onset of insulin resistance and type 2 diabetes in obese persons.

The exact mechanisms by which IL-18 promotes insulin resistance and type 2 diabetes are not fully understood. It may do so by its lipolytic actions that may cause lipidemia and an increase in free fatty acids in the circulation. The cytokine may also interfere with insulin-induced signaling directly as well as indirectly by inducing expression of the suppressors of cytokine signaling proteins.

Importantly, IL-18 and its receptor genes are expressed in human pancreas. The cytokine has also been implicated in causing acute pancreatitis and death of insulin-producing cells[87,88]. A direct role of IL-18 in causing destruction of insulin-producing cells comes from the streptozotocin-induced diabetes in mice. Neutralization of endogenous IL-18 by exogenous administration of IL-18BP-Fc fusion protein relieves hyperglycemia and diabetes[89]. Furthermore, the pancreas becomes larger in size in IL-18 KO mice[77]. Further studies are needed to determine whether the cytokine may cause tissue destruction in the pancreas via Fas/FasL interaction as it does in the liver in septicemia.

Apparently, it seems contradictory that if IL-18 is implicated in the development of insulin resistance, then why do IL-18 KO and IL-18R KO mice become obese and develop insulin resistance. While the exact answer is not known, it may relate to increased appetite, feed intake, lipogenesis, decreased lipolysis, and disturbed energy homeostasis. These observations suggest very strongly that IL-18 may play a contributing role in inducing systemic insulin resistance in HIV-infected patients.

Several observational studies have documented an increased incidence of atherosclerotic lesions as well as of cardiovascular events in HIV-infected patients without any apparent known risk factor[90-93]. It is noteworthy that IL-18 is highly atherogenic. Atherosclerotic lesions begin with infiltration of macrophages and T-cells in subendothelial spaces. Functional IL-18R occur on vascular endothelial and smooth muscle cells[94]. By inducing expression of VCAM-1 on vascular endothelial cells and ICAM-1 on macrophages and T-cells, the cytokine promotes adhesion of these cells to vascular endothelium and their subsequent migration to subendothelial spaces[94].

The cytokine also induces platelet-activating factor and plasminogen activator inhibitor-1 (PAI-1) from the endothelial and other target cells, and promotes activation of platelets and thromboembolic events (reviewed[95,96]). In fact we have previously reported a correlation between platelet activation and increased IL-18 concentrations in HIV-infected patients[97].

Interestingly, IL-18 induces IFNγ expression from vascular smooth muscle cells. The interferon-induced expression of CXCL-16/SR-PSOX on macrophages promotes uptake of oxidized LDL and converts them into lipid-laden "foam cells"[98]. The soluble form of CXCL-16 acts as a chemokine and attracts CXCR6-positive T-cells to the lesion. Administration of IL-18 increases atherosclerosis in aorta and aortic arch twofold in animal models of the disease[99]. Increased levels of the cytokine have been documented in the circulation of the patients suffering from this affliction, and these levels correlate with the severity of the disease[100,101]. Neutralization of this cytokine *in vivo* in animal models of the disease by using IL-18BP has been shown to slow progression of the disease[102]. In addition to increased levels of IL-18, increased concentrations of PAI-1 and enhanced platelet activation have been reported in the circulation of HIV-infected persons[97,103]. Furthermore, increased IL-18 concentrations are associated with plaque instability and myocardial infarction[104]. They are a strong predictor of diabetes-associated nephropathy as well as of death in cardiovascular diseases[105-107]. Increased IL-18 production represents a mechanistic link between psychological stress and increased occurrence of atherosclerosis and ischemic heart[108,109]. Increased concentrations of this cytokine in HIV-infected patients are likely to contribute towards increased incidence of atherosclerotic lesions and cardiovascular events.

HIV-associated dementia, a neurologic complication in HIV-infected persons, is characterized by the development of severe motor and cognitive dysfunctions. Proinflammatory cytokines like IL-18, which are produced in brain in response to HIV infection, play an important role

in the development of HIV-associated dementia in HIV-infected patients (reviewed[110]). They increase the permeability of the blood-brain barrier and promote infiltration of HIV-infected macrophages into the tissue.

Interleukin-18 is an important mediator of communication between nervous, endocrine, and immune systems. Biologically mature forms of the cytokine are produced in response to physical, emotional, and psychological stress or activation of the sympathetic system. Under these conditions, the hypothalamic-pituitary-adrenal axis is stimulated, leading to production of IL-18 from the adrenal cortex. Relaxation, on the other hand, activates the parasympathetic nervous system and inhibits production of this cytokine (reviewed[111]). It is noteworthy that stress-induced IL-18 does not result in enhanced production of IFNγ, as it does not cause increased production of collaborating cytokines like IL-12. The cytokine is also produced in response to these stimuli in the neurohypophysis and several different cell types in the brain, e.g. microglia, ependymal cells, and certain neurons in the medial habenula. Because of its expression in the brain, the cytokine is likely to contribute to neuroinflammation and neurodegeneration by activating microglia. The viral envelope glycoprotein Gp120 has been shown to induce activation of caspase-1 and secretion of IL-1β from a variety of brain cells[110,112]. These cells also express IL-18 and likely produce this cytokine in response to HIV infection. Increased levels of the cytokine have been reported to occur in the cerebrospinal fluid of HIV-infected persons suffering from opportunistic infections of the central nervous system[113]. A role for this cytokine in neurodegeneration is also supported by studies on the brains of Alzheimer patients (reviewed[111]). It may induce enhanced production of reactive oxygen and nitrogen species and cause death of neurons. It may also affect brain function by its direct effects on neurons. It impairs long-term potentiation and N-methyl-D-aspartate receptor-mediated transmission in rat hippocampus[114]. Increased concentrations of the cytokine present in the brain and cerebrospinal fluid of HIV-infected persons may be responsible, at least in part, for dementia and psychological disorders occurring in this infection.

## Conclusions

By its proinflammatory and immune-enhancing properties, IL-18 protects the host from bacterial, fungal, and viral infections. Interleukin-18-induced IFNγ has the potential to inhibit viral replication by inducing an antiviral state in the host cells[115]. Indeed IL-18-induced IFNγ has been shown to protect mice *in vivo* from infections with HSV-1[116]. The interferon could also inhibit HIV replication by antagonizing the effect of Tat[117]. Furthermore, by providing an early source of this interferon, IL-18 may promote development of type 1 antiviral responses. The very fact that many poxviruses encode an IL-18BP-like protein for neutralizing the host IL-18 is strong evidence for an antiviral role of IL-18[20].

A similar antiviral role for IL-18 can be conceived in HIV infections, especially early in the course of the infection. However, the infection is accompanied by compromised production of several other cytokines like IL-12, IL-15, and IL-21[48]. With the decreased/insufficient production of these collaborating cytokines, IL-18 loses its ability to induce IFNγ. A decreased production of IL-18BP may exacerbate the condition. Under these conditions, the ability of IL-18 to promote type 2 responses may predominate. The cytokine may also blunt innate defense mechanisms and rather promote Fas/FasL-mediated tissue destruction. The cytokine may enhance viral replication, cause disturbances in lipid and carbohydrate metabolism and energy homeostasis precipitating HIV-associated lipodystrophy syndrome, insulin resistance, dyslipidemia, cardiovascular manifestations, and dementia in HIV-infected patients.

Clinical studies conducted in this connection in HIV-infected patients also suggest a pathogenic role of the cytokine in HIV-infected individuals. Higher levels of the cytokine were reported to occur in progressors than in nonprogressors. The patients in the latter category maintained these levels near physiological levels[43]. Thus, increased IL-18 concentration in these patients may be acting more as a bane than a boon. The situation may be ameliorated by the use of exogenous cytokines like IL-12, IL-15, or IL-21. This is rather impracticable as the cytokines are highly toxic. Alternately, IL-18 may be neutralized and its induction of Th2-type cytokines may be prevented. The neutralization of this cytokine may also ameliorate the adverse effects of the cytokine on energy and lipid metabolism.

Fortunately, for this purpose, an excellent tool exists in the form of IL-18BP; exogenous infusions of this IL-18 antagonist may be used to modulate IL-18 activity *in vivo* to desirable levels.

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## References


1. Dinarello C. The IL-1 family and inflammatory diseases. Clin Exp Rheumatol. 2002;20:S1-13.
2. Dinarello C. Interleukin-18 and the pathogenesis of inflammatory diseases. Semin Nephrol. 2007;27:98-114.
3. Gracie J, Robertson S, McInnes I. Interleukin-18. J Leukoc Biol. 2003;73:213-24.
4. Sekiyama A, Ueda H, Kashiwamura S, et al. IL-18; a cytokine translates a stress into medical science. J Med Invest. 2005;52:236-9.
5. Sugama S, Wang N, Shimokawa N, et al. The adrenal gland is a source of stress-induced circulating IL-18. J Neuroimmunol. 2006;172:59-65.
6. Ghayur T, Banerjee S, Hugunin M, et al. Caspase-1 processes IFN-[gamma]inducing factor and regulates LPS-induced IFN-[gamma] production. Nature. 1997;386:619-23.
7. Gu Y, Kuida K, Tsutsui H, et al. Activation of interferon-gamma inducing factor mediated by interleukin-1beta converting enzyme. Science. 1997;275:206-9.
8. Shaw M, Reimer T, Kim Y-G, Nuñez G. NOD-like receptors: bona fide intracellular microbial sensors. Curr Opin Immunol. 2008;20:377-82.
9. Sirard J-C, Vignal C, Dessein R, Chamaillard M. Nod-like receptors: Cytosolic watchdogs for immunity against pathogens. PLoS Pathogens. 2007;3:e152.
10. Halle A, Hornung V, Petzold G, et al. The NALP3 inflammasome is involved in the innate immune response to amyloid-[beta]. Nat Immunol. 2008;9:857-65.
11. Hewinson J, Moore S, Glover C, Watts A, MacKenzie A. A key role for redox signaling in rapid P2X7 receptor-induced IL-1 beta processing in human monocytes. J Immunol. 2008;180:8410-20.
12. Mariathasan S, Weiss D, Newton K, et al. Cryopyrin activates the inflammasome in response to toxins and ATP. Nature. 2006;440:228-32.
13. Ozoren N, Masumoto J, Franchi L, et al. Distinct roles of TLR2 and the adaptor ASC in IL-1beta/IL-18 secretion in response to Listeria monocytogenes. J Immunol. 2006;176:4337-42.
14. Perregaux D, McNiff P, Laliberte R, Conklyn M, Gabel C. ATP acts as an agonist to promote stimulus-induced secretion of IL-1beta and IL-18 in human blood. J Immunol. 2000;165:4615-23.
15. Kumar S, McDonnell P, Lehr R, et al. Identification and initial characterization of four novel members of the interleukin-1 family. J Biol Chem. 2000;275:10308-14.
16. Novick D, Kim S, Fantuzzi G, Reznikov L, Dinarello C, Rubinstein M. Interleukin-18 binding protein: a novel modulator of the Th1 cytokine response. Immunity. 1999;10:127-36.
17. Novick D, Schwartsburd B, Pinkus R, et al. A novel IL-18BP ELISA shows elevated serum IL-18BP in sepsis and extensive decrease of free IL-18. Cytokine. 2001;14:334-42.
18. Hurgin V, Novick D, Rubinstein M. The promoter of IL-18 binding protein: activation by an IFN-gamma-induced complex of IFN regulatory factor 1 and CCAAT/enhancer binding protein beta. PNAS. 2002;99:16957-62.
19. Paulukat J, Bosmann M, Nold M, et al. Expression and release of IL-18 binding protein in response to IFN-gamma. J Immunol. 2001;167:7038-43.
20. Reading P, Smith G. Vaccinia virus interleukin-18-binding protein promotes virulence by reducing gamma interferon production and natural killer and T-cell activity. J Virol. 2003;77:9960-8.
21. Xiang Y, Moss B. IL-18 binding and inhibition of interferon gamma induction by human poxvirus-encoded proteins. PNAS. 1999;96:11537-42.
22. Banda N, Vondracek A, Kraus D, et al. Mechanisms of inhibition of collagen-induced arthritis by murine IL-18 binding protein. J Immunol. 2003;170:2100-5.
23. Nakamura K, Okamura H, Wada M, Nagata K, Tamura T. Endotoxin-induced serum factor that stimulates gamma interferon production. Infect Immun. 1989;57:590-5.
24. Gutzmer R, Langer K, Mommert S, Wittmann M, Kapp A, Werfel T. Human dendritic cells express the IL-18R and are chemoattracted to IL-18. J Immunol. 2003;171:6363-71.
25. Morel J, Park C, Woods J, Koch A. A novel role for interleukin-18 in adhesion molecule induction through NFkappa B and phosphatidylinositol 3-kinase-dependent signal transduction pathways. J Biol Chem. 2001;276:37069-75.
26. Walker W, Aste-Amezaga M, Kastelein R, Trinchieri G, Hunter C. IL-18 and CD28 use distinct molecular mechanisms to enhance NK cell production of IL-12-induced IFN-gamma. J Immunol. 1999;162:5894-901.
27. Okamura H, Tsutsui H, Kashiwamura S, Yoshimoto T, Nakanishi K. Interleukin18: a novel cytokine that augments both innate and acquired immunity. Adv Immunol. 1998;70:281-312.
28. Okamura H, Nagata K, Komatsu T, et al. A novel co-stimulatory factor for gamma interferon induction found in the livers of mice causes endotoxic shock. Infect Immun. 1995;63:3966-72.
29. Okamura H, Tsutsui H, Komatsu T, et al. Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature. 1995;378:88-91.
30. Harrington L, Hatton R, Mangan P, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol. 2005;6:1123-32.
31. Weaver C, Hatton R, Mangan P, Harrington L. IL-17 family cytokines and the expanding diversity of effector T cell lineages. Ann Rev Immunol. 2007;25:821-52.
32. Yoshimoto T, Nakanishi K. Roles of IL-18 in basophils and mast cells. Allergol Int. 2006;55:105-13.
33. Hata H, Yoshimoto T, Hayashi N, Hada T, Nakanishi K. IL-18 together with anti-CD3 antibody induces human Th1 cells to produce Th1-and Th2cytokines and IL-8. Int Immunol. 2004;16:1733-9.
34. Sugimoto T, Ishikawa Y, Yoshimoto T, Hayashi N, Fujimoto J, Nakanishi K. Interleukin 18 acts on memory T helper cells type 1 to induce airway inflammation and hyperresponsiveness in a naive host mouse. J Exp Med. 2004;199:535-45.
35. Dao T, Ohashi K, Kayano T, Kurimoto M, Okamura H. Interferon-gamma-inducing factor, a novel cytokine, enhances Fas ligand-mediated cytotoxicity of murine T helper 1 cells. Cell Immunol. 1996;173:230-5.
36. Tsutsui H, Nakanishi K, Matsui K, et al. IFN-gamma-inducing factor upregulates Fas ligand-mediated cytotoxic activity of murine natural killer cell clones. J Immunol. 1996;157:3967-73.
37. Tsutsui H, Matsui K, Okamura H, Nakanishi K. Pathophysiologic roles of interleukin-18 in inflammatory liver diseases. Immunol Rev. 2000;174: 192-209.
38. Ahmad R, Sindhu S, Toma E, Morisset R, Ahmad A. Elevated levels of circulating interleukin-18 in HIV-infected individuals: Role of peripheral blood mononuclear cells and implications for AIDS pathogenesis. J Virol. 2002;76:12448-56.
39. Torre D, Speranza F, Martegani R, et al. Circulating levels of IL-18 in adult and pediatric patients with HIV-1 infection. AIDS. 2000;14: 2211-12.
40. Torre D, Pugliese A. Interleukin-18: a proinflammatory cytokine in HIV-1 infection. Curr HIV Res. 2006;4:423-30.
41. Sailer C, Pott G, Dinarello C, et al. Whole-blood interleukin-18 level during early HIV-1 infection is associated with reduced CXCR4 coreceptor expression and interferon-gamma levels. J Infect Dis. 2007;195:734-8.
42. David D, Chevrier D, Treilhou M, et al. IL-18 underexpression reduces IL-2 levels during HIV infection: a critical step towards the faulty cell-mediated immunity? AIDS. 2000;14:2212-14.
43. Stylianou E, Bjerkeli V, Yndestad A, et al. Raised serum levels of interleukin18 is associated with disease progression and may contribute to virologic treatment failure in HIV-1-infected patients. Clin Exp Immunol. 2003;132:462-6.
44. Song W, Wilson C, Allen S, et al. Interleukin 18 and human immunodeficiency virus type I infection in adolescents and adults. Clin Exp Immunol. 2006;144:117-24.
45. Ahmad A, Ahmad R, Toma E, Morisset R, Menezes J. Impaired induction of IL-15 in response to herpes simplex virus type 1 infection in peripheral blood mononuclear cells of HIV-infected patients. AIDS. 2000;14:744-6.
46. Chambers K, Parato K, Angel J. Active cellular infection of myeloid cells is required for HIV-1-mediated suppression of interleukin-12 p40 expression. Cell Immunol. 2002;215:120-32.
47. Chehimi J, Starr S, Frank I, et al. Impaired interleukin 12 production in human immunodeficiency virus-infected patients. J Exp Med. 1994;179:1361-6.
48. Iannello A, Debbeche O, Samarani S, Ahmad A. Antiviral NK cell responses in HIV infection: II. viral strategies for evasion and lessons for immunotherapy and vaccination. J Leukoc Biol. 2008;84:27-49.
49. Clerici M, Hakim F, Venzon D, et al. Changes in interleukin-2 and interleukin4 production in asymptomatic, HIV-seropositive individuals. J Clin Invest. 1993;91:759-65.
50. Meyaard L, Hovenkamp E, Keet I, et al. Single cell analysis of IL-4 and IFNgamma production by T cells from HIV-infected individuals: decreased IFNgamma in the presence of preserved IL-4 production. J Immunol. 1996;157:2712-18.
51. Shearer G, Clerici M. Cytokine profiles in HIV type 1 disease and protection. AIDS Res Hum Retroviruses. 1998;14:149-52.
52. Mazodier K, Marin V, Novick D, et al. Severe imbalance of IL-18/IL-18BP in patients with secondary hemophagocytic syndrome. Blood. 2005;106:3483-9.
53. Shibatomi K, Ida K, Yamasaki S, et al. A novel role for interleukin-18 in human natural killer cell death: High serum levels and low natural killer cell numbers in patients with systemic autoimmune diseases. Arthritis Rheum. 2001;44:884-92.
54. Iannello A, Samarani S, Debbeche O, et al. Potential role of IL-18 in the immunopathogenesis of AIDS: Involvement in fratricidal killing of NK cells. J Virol. 2009;83:5999-6010.
55. Kaplan D, Sieg S. Role of the Fas/Fas ligand apoptotic pathway in HIV type 1 disease. J. Virol. 1998;72:6279-82.
56. Klein S, Klebba C, Kauschat D, et al. Interleukin-18 stimulates HIV-1 replication in a T-cell line. Eur Cytokine Netw. 2000;11:47-52.
57. Pugliese A, Gennero L, Vidotto V, Speranza F, Tambini R, Torre D. Interleukin-18 enhances HIV-1 production in a human chronically-infected T cell line (H9-V). Cell Biochem Funct. 2002,20:333-7.
58. Shapiro L, Puren A, Barton H, et al. Interleukin 18 stimulates HIV type 1 in monocytic cells. PNAS. 1998;95:12550-5.
59. Choi H, Dinarello CA, Shapiro L. Interleukin-18 inhibits HIV type 1 production in peripheral blood mononuclear cells. J Infect Dis. 2001;184:560-8.

60. Allen J, Wong H, Guyre P, Simon G, Wahl S. Association of circulating receptor Fc gamma RIII-positive monocytes in AIDS patients with elevated levels of transforming growth factor-beta. J Clin Invest. 1991;87:1773-9.
61. Iannello A, Tremblay C, Routy J, Boulassel M, Toma E, Ahmad A. Decreased levels of circulating IL-21 in HIV-infected AIDS patients: Correlation with CD4+ T cell counts. Viral Immunol. 2008;21:385-8.
62. Parato K, Kumar A, Badley A, et al. Normalization of natural killer cell function and phenotype with effective anti-HIV therapy and the role of IL10. AIDS. 2002;16:1251-6.
63. Kaizu M, Ami Y, Nakasone T, et al. Higher levels of IL-18 circulate during primary infection of monkeys with a pathogenic SHIV than with a nonpathogenic SHIV. Virology. 2003;313:8-12.
64. Senpuku H, Asano T, Salam A, et al. Effects of human interleukin-18 and interleukin-12 treatment on human lymphocyte engraftment in NOD-scid mouse. Immunology. 2002;107:232-42.
65. de Ferranti S, Mozaffarian D. The perfect storm: Obesity, adipocyte dysfunction, and metabolic consequences. Clin Chem. 2008;54:945-55.
66. Villarroya F, Domingo P, Giralt M. Lipodystrophy in HIV 1-infected patients: lessons for obesity research. Int J Obes. 2007;31:1763-9.
67. Wood I, Wang B, Jenkins J, Trayhurn P. The pro-inflammatory cytokine IL18 is expressed in human adipose tissue and strongly upregulated by TNF-alpha in human adipocytes. Biochem Biophys Res Commun. 2005;337:422-9.
68. Carr A, Samaras K, Burton S, et al. A syndrome of peripheral lipodystrophy, hyperlipidemia and insulin resistance in patients receiving HIV protease inhibitors. AIDS. 1998;12:F51-8.
69. Kotler D, Ionescu G, Johnson J, et al. Studies of adipose tissue metabolism in human immunodeficiency virus and associated lipodystrophy. Clin Infect Dis. 2003;37:S47-51.
70. Domingo P, Matias-Guiu X, Pujol R, et al. Subcutaneous adipocyte apoptosis in HIV-1 protease inhibitor-associated lipodystrophy. AIDS. 1999;13:2261-7.
71. Lindegaard B, Hansen A, Gerstoft J, Pedersen B. High plasma level of interleukin-18 in HIV-infected subjects with lipodystrophy. J Acquir Immune Defic Syndr. 2004;36:588-93.
72. Lindegaard B, Hansen A, Pilegaard H, et al. Adipose tissue expression of IL18 and HIV-associated lipodystrophy. AIDS. 2004;18:1956-8.
73. Chandrasekar B, Patel D, Mummidi S, Kim J, Clark R, Valente A. Interleukin-18 suppresses adiponectin expression in 3T3-L1 adipocytes via a novel signal transduction pathway involving ERK1/2-dependent NFATc4 phosphorylation. J Biol Chem. 2008;283:4200-9.
74. Bastard J, Maachi M, Lagathu C, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw. 2006;17:4-12.
75. Herder C, Schneitler S, Rathmann W, et al. Low-grade inflammation, obesity, and insulin resistance in adolescents. J Clin Endocrinol Metab. 2007;92:4569-74.
76. Olusi S, Al-Awadhi A, Abraham M. Relations of serum interleukin 18 levels to serum lipid and glucose concentrations in an apparently healthy adult population. Horm Res. 2003;60:29-33.
77. Netea M, Joosten L, Lewis E, et al. Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. Nat Med. 2006;12:650-6.
78. Oikawa Y, Shimada A, Kasuga A, et al. Systemic administration of IL-18 promotes diabetes development in young nonobese diabetic mice. J Immunol. 2003;171:5865-75.
79. Deng J, Hua K, Lesser S, et al. Interleukin-4 mediates STAT6 activation in 3T3-L1 preadipocytes but not adipocytes. Biochem Biophysl Res Commun. 2000;267:516-20.
80. Hadigan C, Miller K, Corcoran C, et al. Fasting hyperinsulinemia and changes in regional body composition in HIV-infected women. J Clin Endocrinol Metab. 1999;84:1932-7.
81. Stein J. Managing cardiovascular risk in patients with HIV infection. J Acquir Immune Defic Syndr. 2005;38:115-23.
82. Falasca K, Manigrasso M, Racciatti D, et al. Associations between hypertriglyceridemia and serum ghrelin, adiponectin, and IL-18 levels in HIV-infected patients. Ann Clin Lab Sci. 2006;36:59-66.
83. Falasca K, Ucciferri C, Manzoli L, et al. Metabolic syndrome and cardiovascular risk in HIV-infected patients with lipodystrophy. Int J Immunopathol Pharmacol. 2007;20:519-27.
84. King G. The role of inflammatory cytokines in diabetes and its complications. J Periodontol. 2008;79:1527-34.
85. Thorand B, Kolb H, Baumert J, et al. Elevated levels of interleukin-18 predict the development of type 2 diabetes: results from the MONICA/KORA Augsburg study, 1984-2002. Diabetes. 2005;54:2932-8.
86. Medina-Gomez G, Gray S, Vidal-Puig A. Adipogenesis and lipotoxicity: role of peroxisome proliferator-activated receptor gamma (PPARgamma) and PPARgammacoactivator-1 (PGC1). Public Health Nutr. 2007;10:1132-7.
87. Schneider A, Haas S, Hildenbrand R, et al. Enhanced expression of interleukin18 in serum and pancreas of patients with chronic pancreatitis. World J Gastroenterol. 2006;12:6507-14.
88. Sennello J, Fayad R, Pini M, et al. Interleukin-18, together with interleukin-12, induces severe acute pancreatitis in obese but not in nonobese leptin-deficient mice. PNAS. 2008;105:8085-90.
89. Nicoletti F, Di Marco R, Papaccio G, et al. Essential pathogenic role of endogenous IL-18 in murine diabetes induced by multiple low doses of streptozotocin. Prevention of hyperglycemia and insulitis by a recombinant IL-18-binding protein: Fc construct. Eur J Immunol. 2003;33: 2278-86.
90. Mangili A, Gerrior J, Tang AM, et al. Risk of cardiovascular disease in a cohort of HIV-infected adults: A study using carotid intima-media thickness and coronary artery calcium score. Clin Infect Dis. 2006;43: 1482-9.
91. McComsey G, O'Riordan M, Hazen S, et al. Increased carotid intima media thickness and cardiac biomarkers in HIV infected children. AIDS. 2007;21:921-7.
92. Tabib A, Greenland T, Mercier I, Loire R, Mornex J. Coronary lesions in young HIV-positive subjects at necropsy. Lancet. 1992;340:730.
93. Constans J, Marchand J, Conri C, et al. Asymptomatic atherosclerosis in HIV-positive patients: A case-control ultrasound study. Ann Med. 1995;27:683-5.
94. Gerdes N, Sukhova GK, Libby P, et al. Expression of interleukin (IL)-18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells, and macrophages: Implications for artherogenesis. J Exp Med. 2002;195:245-57.
95. Dinarello C. The IL-1 family and inflammatory diseases. Clin Exp Rheumatol. 2005;2002:S1-13.
96. Wang M, Markel T, Meldrum D. Interleukin 18 in the heart. Shock. 2008; 30:3-10.
97. Ahmad R, Iannello A, Samarani S, et al. Contribution of platelet activation to plasma IL-18 concentrations in HIV-infected AIDS patients. AIDS. 2006;20:1907-9.
98. Wagsater D, Olofsson P, Norgren L, Stenberg B, Sirsjo A. The chemokine and scavenger receptor CXCL16/SR-PSOX is expressed in human vascular smooth muscle cells and is induced by interferon gamma. Biochem Biophys Res Commun. 2004;325:1187-93.
99. Whitman S, Ravisankar P, Daugherty A. Interleukin-18 enhances atherosclerosis in apolipoprotein E-/-mice through release of interferon gamma. Circ Res. 2002;90:e34-8.
100. Aso Y, Okumura K, Takebayashi K, Wakabayashi S, Inukai T. Relationships of plasma interleukin-18 concentrations to hyperhomocysteinemia and carotid intimal-media wall thickness in patients with type 2 diabetes. Diabetes Care. 2003;26:2622-7.
101. Yamagami H, Kitagawa K, Hoshi T, et al. Associations of serum IL-18 levels with carotid intima-media thickness. Arterioscler Thromb Vasc Biol. 2005;25:1458-62.
102. Mallat Z, Corbaz A, Scoazec A, et al. Interleukin-18/Interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability. Circ Res. 2001;89:e41-5.
103. He G, Andersen S, Haugaard, et al. Plasminogen activator inhibitor type 1 (PAI-1) in plasma and adipose tissue in HIV-associated lipodystrophy syndrome. Implications of adipokines. Eur J Clin Invest. 2005;35:583-90.
104. Mallat Z, Corbaz A, Scoazec A, et al. Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. Circulation. 2001;104:1598-603.
105. Araki S, Haneda M, Koya D, et al. Predictive impact of elevated serum level of IL-18 for early renal dysfunction in type 2 diabetes: an observational follow-up study. Diabetologia. 2007;50:867-73.
106. Blankenberg S, Tiret L, Bickel C, et al. Interleukin-18 is a strong predictor of cardiovascular death in stable and unstable angina. Circulation. 2002;106:24-30.
107. Nakamura A, Shikata K, Hiramatsu M, et al. Serum interleukin-18 levels are associated with nephropathy and atherosclerosis in Japanese patients with type 2 diabetes. Diabetes Care. 2005;28:2890-5.
108. Vale S. Effect of high-dose vitamin E on insulin resistance and associated parameters in overweight subjects: Response to Manning, et al. Diabetes Care. 2005;28:230-1.
109. Yan L, Liu K, Matthews KA, et al. Psychosocial factors and risk of hypertension: The coronary artery risk development in young adults (CARDIA) study. JAMA. 2003;290:2138-48.
110. Brabers N, Nottet H. Role of the pro-inflammatory cytokines TNF-alpha; and IL-1beta; in HIV-associated dementia. Eur J Clin Invest. 2006;36: 447-58.
111. Sugama S, Conti B. Interleukin-18 and stress. Brain Res Rev. 2008;58: 85-95.
112. Simi A, Lerouet D, Pinteaux E, Brough D. Mechanisms of regulation for interleukin-1-beta in neurodegenerative disease. Neuropharmacology. 2007;52:1563-9.
113. von Giesen H, Jander S, Köller H, Arendt G. Serum and cerebrospinal fluid levels of interleukin-18 in HIV-1-associated central nervous system disease. J Neurovirol. 2004;10:383-6.
114. Curran B, O'Connor J. The pro-inflammatory cytokine interleukin-18 impairs long-term potentiation and NMDA receptor-mediated transmission in the rat hippocampus in vitro. Neuroscience. 2001;108:83-90.
115. Guidotti L, Chisari F. Non-cytolytic control of viral infections by the innate and adaptive immune response Ann Rev Immunol. 2001;19:65-91.
116. Fujioka N, Akazawa R, Ohashi K, Fujii M, Ikeda M, Kurimoto M. Interleukin-18 protects mice against acute herpes simplex virus type 1 infection. J Virol. 1999;73:2401-9.
117. Sarol L, Imai K, Asamitsu K, Tetsuka T, Barzaga N, Okamoto T. Inhibitory effects of IFN-gamma on HIV-1 replication in latently infected cells. Biochem Biophys Res Commun. 2002;291:890-6.

## 4- Les cellules Natural Killer, leur biologie et leurs déterminants génétiques dans la résistance au VIH-1

Les lymphocytes Natural Killer (NK) sont des cellules de l'immunité innée intervenant en première ligne dans la lutte contre divers agents pathogènes et dans la surveillance des tumeurs. Les informations détaillées concernant leur biologie et l'impact de leur immunogénétique dans la progression du SIDA ont fait l'objet d'un article de revue. Cet article a été soumis à un processus de révision par les pairs avant sa publication.

**Article 4 :** **Iannello A**, Debbeche O, Samarani S, and Ahmad A. Antiviral NK Cell Responses in HIV Infection: I. NK cell receptor genes as determinants of HIV resistance and progression to AIDS. 2008. ***Journal of Leukocyte Biology*** **84(1): 1-26**.

**Résumé :** Les cellules NK jouent un rôle important dans le contrôle des infections virales. Elles peuvent tuer les cellules infectées de façon directe aussi bien que par ADCC. Elles sécrètent une multitude de facteurs solubles et interagissent directement avec d'autres cellules du système immunitaire comme les cellules dendritiques. Les cellules NK expriment une série de récepteurs activateurs et inhibiteurs qui se lient à leurs ligand respectifs exprimés sur la surface des cellules cibles. Ces ligands représentent les antigènes du CMH-I classiques et non classiques, les protéines de type CMH-I et une variété d'autres molécules du soi ou dérivées de protéines virales. Les récepteurs de la famille des *killer-cell Ig-like* (KIR) sont très polymorphiques comme c'est le cas pour leurs ligands (CMH-I). Plusieurs études récentes ont démontré que certaines interactions épistatiques entre les KIR et les gènes du CMH-I peuvent déterminer la résistance innée de l'hôte à des infections virales. Dans cette article de revue, nous discutons des dernières découvertes concernant l'immunobiologie des cellules NK et décrivons comment les gènes codant pour les récepteurs de ces cellules, seuls et en combinaison avec les gènes codant pour le CMH-I, peuvent déterminer la résistance ou la susceptibilité génétique à une infection par le VIH-1 et au développement du SIDA.

# Article 4

**Iannello A**, Debbeche O, Samarani S, and Ahmad A. Antiviral NK Cell Responses in HIV Infection: I. NK cell receptor genes as determinants of HIV resistance and progression to AIDS. 2008. ***Journal of Leukocyte Biology*** **84(1): 1-26**.

# Antiviral NK cell responses in HIV infection: I. NK cell receptor genes as determinants of HIV resistance and progression to AIDS

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**Abstract: NK cells play an important role in controlling viral infections. They can kill virus-infected cells directly as well as indirectly via antibody-dependent, cell-mediated cytotoxicity. They need no prior sensitization and expansion for this killing. NK cells are also considered as important regulators of antiviral immune responses. They do so by secreting a multitude of soluble mediators and by directly interacting with other immune cells, e.g., dendritic cells. NK cells do not possess a single well-defined receptor to recognize antigens on target cells. Instead, they express an array of inhibitory and activating receptors and coreceptors, which bind to their cognate ligands expressed on the surface of target cells. These ligands include classical and nonclassical MHC class I antigens, MHC-like proteins, and a variety of other self- and virus-derived molecules. They may be expressed constitutively and/or de novo on the surface of virus-infected cells. NK cell receptors (NKRs) of the killer-cell Ig-like receptor (KIR) family, like their MHC class I ligands, are highly polymorphic. Several recent studies suggest that epistatic interactions between certain *KIR* and *MHC class I* genes may determine innate resistance of the host to viral infections, including HIV. In the first part of this review article, we provide an overview of the current state of knowledge of NK cell immunobiology and describe how NKR genes, alone and in combination with HLA genes, may determine genetic resistance/susceptibilty to HIV infection and the development of AIDS in humans. *J. Leukoc. Biol.* 84: 1–26; 2008.**

***Key Words:*** *CD94/NKG2 · chemokines · cytokines · HIV-1 · HLA · KIR · KIR haplotypes · MHC class I · MICA · MICB · NKG2D · ULBP*

## INTRODUCTION

HIV is the causative agent of AIDS: a dreadful and socially stigmatizing disease syndrome of the 20th century that inevitably leads to death. The AIDS epidemic has killed more than 25 million humans since 1981. At present, close to 40 million people are infected with the virus in the world. It has been estimated that in 2006, more than 4 million new infections occurred with the virus, and 3 million people died of AIDS in the world. It is widely believed that the viral infection is spreading in several underdeveloped and developing countries, e.g., China, India, and Eastern European countries (www.unaids.org). This situation underlines the need for developing an effective anti-HIV vaccine. However, at present, the prospects of developing an effective anti-HIV vaccine do not seem very promising. Although, anti-HIV drugs have significantly prolonged lifespans of HIV-infected patients and have reduced deaths, they do not cure the infection. Furthermore, sooner or later, the virus develops resistance to these drugs. Researchers have made spectacular advances in studying and analyzing immune responses to the virus. Most of these studies have largely focused on virus-specific immunity, i.e., HIV-specific antibodies, $CD4^+$ and $CD8^+$ T cell responses. It is noteworthy that the inductive and effector mechanisms of these responses depend on the recognition of small viral peptides by the immune cells. Unfortunately, small RNA viruses (such as HIV) are quite apt in neutralizing these responses by their remarkable ability to mutate (reviewed in ref. [1]). On the other hand, innate immune responses do not limit themselves to the recognition of small viral peptides. Instead, innate immune cells recognize so-called molecular patterns associated with pathogens (e.g., dsRNA, CpG motifs in viral DNA), altered self (reduced expression of MHC antigens), and/or induced self (de novo expression of pathogen-induced host proteins) on the body's own cells. Activation of innate immune cells is also a prerequisite for mounting an effective, pathogen-specific adaptive immunity. Understanding these responses may provide better ways of controlling viral infections.

NK cells constitute an important component of the host's innate immune system. Once considered as relatively unimportant and nonspecific killers of tumor cells, NK cells are now recognized as important cells with ready-to-go effector and regulatory functions. For long, NK cells have been known to

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kill virus-infected cells, and NK cell-deficient individuals have been known to suffer from repeated viral and bacterial infections [2, 3]. However, a lack of understanding of NK cell immunobiology until recent years has been an impediment in appreciating the role of these cells in controlling these infections. Today, scientists have made significant advances in understanding how NK cells function and regulate innate and adaptive immune responses. Consequently, we have learned a lot about the role of these cells in HIV and other viral infections. The review focuses on NK cell responses in HIV infections and their relevance to anti-HIV resistance, immunotherapy, and vaccination. We also underline some of the important unresolved issues with respect to these cells in HIV infection that need to be addressed in future research. Understandably, it will have to begin with an overview of the current immunobiology of NK cells.

## NK CELLS

NK cells are a population of low-density, large granular lymphocytes, which mainly develop and differentiate in bone marrow and then enter into the circulation. Significant numbers of NK cells also develop and differentiate in thymus, spleen, tonsils, and lymph nodes [4, 5]. As a result of their different sites and pathways of development, NK cells are heterogeneous with respect to their phenotypic characteristics and functional capabilities. They make up 10–15% of the PBMC. In response to proinflammatory stimuli, which may be induced by a viral infection, NK cells migrate to various tissues and organs of the body. Significant numbers of NK cells occur in thymus, lymph nodes, gut, liver, peritoneum, and other organs and tissues of the body. NK cells represent a cell lineage distinct from those of monocytes, granulocytes, and B cells. They share a common hematopoietic progenitor with T cells. However, they are less specialized than T cells and retain certain ancestral characteristics of plasticity and versatility. Phenotypically, NK cells are $CD3^-$, $CD2^+$, $CD16^+$, $CD56^+$, $CD14^-$, and $CD19^-$. Unlike T and B cells, they do not express a well-defined single antigen receptor. They also do not express recombinase activating genes (RAG) and retain their TCR and BCR genes in germ-line configurations. See **Table 1** for commonly used NK cell markers. It is noteworthy that most of these markers are not NK cell-specific, as cells of other types may also express them. However, NKp46 and NKp30 may be considered as NK cell-specific markers, as non-NK cells rarely express them.

NK cells should not be confused with NKT cells. The two cell types are quite distinct from each other with respect to their origin, phenotype, antigen recognition, and certain effector functions (reviewed in refs. [6, 7]). As their name implies, NKT cells share characteristics with NK and T cells; i.e., they are $CD3^+$, bear clonotypic $\alpha\beta$ TCR and certain NK cell markers ($CD56^+$, $CD161^+$), and secrete IFN-$\gamma$ and/or IL-4. They recognize self and foreign glycolipids in association with a MHC class I-related glycoprotein CD1d. Activation of NKT cells usually leads to activation of NK cells and dendritic cells (DC) in the body.

Human NK cells can be divided into two major subsets based on the level of expression of CD56 and the presence or absence of CD16. The two markers are usually expressed reciprocally on these cells. The two subsets $CD56^{high}CD16^-$ and $CD56^{low}CD16^+$ represent ~10% and 90% of NK cells present in the peripheral blood, respectively [8, 9]. The cells in the two subsets differ in their proliferative potential, homing characteristics, functional capabilities, and responses to different cytokines (listed in **Table 2**). The cells in the former subset express high-affinity receptors for IL-2, proliferate in response to picomolar concentrations of the cytokine, produce mainly cytokines upon activation, and have low cytotoxic potential. They express little KIR and preferentially migrate to secondary lymphoid organs, e.g., lymph nodes, tonsils. Most of the NK cells in lymph nodes are $CD56^{high}$. The $CD56^{low}$ $CD16^+$ NK cells express low-affinity IL-2Rs, proliferate in response to nanomolar concentrations of IL-2, express KIR, and are highly cytotoxic. These NK cells migrate to inflamed tissues in response to chemotactic stimuli. By virtue of expression of CD16, they are also efficient mediators of ADCC. The $CD56^{high}$ subset is less cytotoxic as compared with the $CD56^{low}$ subset, probably as a result of their lower expression of perforin and $\zeta$-chain [9, 10]. The $\zeta$-chain is a signal-transducing component of the high-affinity receptor for IgE (Fc$\varepsilon$RI) and acts as a signaling partner for several activating NK cell receptors

TABLE 1. Common Markers Used for Phenotypic Characterization of Human NK Cells

| Marker | Structure | Ligand | Prevalence | Function |
|---|---|---|---|---|
| CD2 | Ig-like | CD58 | All | Adhesion, Conjugate formation |
| CD3 | Ig-like | TCR | Absent | – |
| CD19 | Ig-like | BCR | Absent | – |
| CD14 | G-LRR | LPS | Absent | – |
| CD16A | Ig-like | IgG[a] | Subset | ADCC, Degranulation |
| CD56 | Ig-like | Self | Subset | Homotypic adhesion |
| NKp46[b] | Ig-like | HA | All | Activation |
| NKp44[b] | Ig-like | HA | All | Activation |
| NKp30[b] | Ig-like | ? | All | Activation |
| CD161 | C-type Lectin | LLT-1 | All | Activation? |
| CD8 | Ig-like | MHC class I | Subset | Costimulation, Conjugate formation |

Prevalence refers to whether all or a subset of NK expresses the marker. G-LRR, GPI-anchored leucine-rich repeat-containing glycoprotein; HA, hemagglutinin of influenza virus; LLT-1, lectin-like transcript-1; ADCC, antibody-dependent, cell-mediated cytotoxicity. [a] IgG, IgG aggregated or found in immune complexes. [b] The marker is specific to NK cells. ? indicates unknown or controversial.

TABLE 2. Characteristics of Two Major NK Cell Subsets

| Characteristic | $CD56^{hi}CD16^{low}$ | $CD56^{low}CD16^{hi}$ |
|---|---|---|
| IL-2R | High affinity | Low affinity |
| Cytotoxicity | + | +++ |
| Perforin | Low | High |
| Cytokine production[a] | +++ | + |
| Main location | Lymph nodes[b] | Blood |
| KIR | Low | High |
| NKG2 Expression | High | Low |
| ADCC Function | Inefficient | Efficient |
| LFA-1 | Low | High |
| CD62L[c] | High | Low |
| CCR7[d] | High | Low |
| SHIP-1[e] | High | Low |
| CD3ζ-chain[f] | Low | High |
| IL-7R | High | Low |
| c-Kit[g] | High | Low |
| ICAM-3 | High | Low |
| Lysozyme production | Yes | No |

High and low refer to levels of expression. [a] Cytokines include IFN-γ, TNF-α, TNF-β, IL-5, IL-13, and GM-CSF. [b] In T cell-rich areas of lymph nodes and other secondary lymphoid organs and in body tissues and organs such as liver. [c,d] Lymph node homing receptors. [e] Needed for IFN-γ production. [f] Signaling partner for activating receptors such as CD16a. [g] c-Kit receptor tyrosine kinase needed for IL-mediated proliferation. KIR, killer-cell Ig-like receptors; NKG2, NK cell group 2; CD62L, CD62 ligand.

(NKRs), e.g., CD16a, NKp30, NKp46, etc. Both NK cell subsets become potent killer cells upon incubation with cytokines and are called lymphokine-activated killer cells. Some workers also differentiate between $CD16^{high}$ and $CD16^{dim}$ subsets of NK cells. Furthermore, NK cells expressing CD56 and CD16 have also been described. They may represent different activation and differentiation states of NK cells. It is noteworthy that incubation with different cytokines may change phenotypic, functional, as well as homing characteristics of NK cells. For example, IL-2, IL-12, and IL-15 can convert $CD56^{high}CD16^{-}$ and $CD56^{low}CD16^{+}$ NK cell subsets into $CD56^{bright}CD16^{+}$ cells. On the other hand, TGF-β1 converts $CD16^{+}$ NK cells into $CD16^{-}$ NK cells [11–13].

## NK CELL FUNCTIONS

Although NK cells have been traditionally known for their ability to kill certain tumor and virus-infected cells, they also perform other important roles (detailed in **Table 3**). Like neutrophils, NK cells can kill bacteria by secreting α-defensins: the antimicrobial peptides that can disrupt bacterial cell walls. They act as important immunoregulatory cells by secreting cytokines and chemokines. It is noteworthy that NK cells are the only known source of IFN-γ other than activated T cells. The cytokine is known to activate macrophages and drive $CD4^{+}$ T cell differentiation into type 1 (TH1) cells. It also induces expression of TRAIL on T cells. An immediate release of this cytokine from NK cells in early stages of an infection is crucial for inducing virus-specific immunity. In addition to IFN-γ, NK cells have been documented to secrete TNF-α, GM-CSF, IL-5, IL-13, IL-10, TGF-β, MIP-1α, MIP-1β, RANTES, NO, etc. (reviewed in ref. [14]). In addition to their immunoregulatory properties, IFN-γ and TNF-α can induce an antiviral state in the host cells and inhibit virus replication by noncytopathic mechanisms. This virtual curing of the infected cells is increasingly being appreciated in controlling viral infections [15].

NK cells regulate immune responses by interacting physically with other cells in the body. Their interactions with monocyte-macrophages, DC, and T, B, NKT, and vascular endothelial cells are beginning to be understood. These interactions bear important implications for viral infections, malignancy, and autoimmunity. Under appropriate conditions, NK cells can cause differentiation of monocytes into macrophages [16]. They also interact with activated macrophages. These interactions result into activation of interacting NK cells via 2B4 and CD48 interactions. NK cells also kill macrophages that become overactive (with high doses of LPS) and express de novo ligands for certain activating NKRs. They also kill macrophages, which produce immunosuppressive, cytokine-like TGF-β and IL-10. Consequently, NK cells promote TH1 responses needed for the induction of an effective immune response against intracellular pathogens [17, 18]. Interestingly, NK cells themselves have the potential to act as APC. It may be relevant to mention here that a special kind of cell was described in mice with DC and NK cell-type characteristics. It was named as IFN-producing killer DC (IKDC), which kill virus-infected cells as well as tumor cells, produce IFNs, and present antigens to T cells [19–21]. The IKDC, in fact, represent a distinct activation stage of NK cells [22]. Such cells have not been found in humans. However, it is argued that the human immune system is more developed than that of mice, and all human NK cells can act as APC under appropriate conditions. Activated human NK cells express several molecules, which enable them to interact with T cells, e.g., CD11c, MHC class II, CD80, CD86, CCR7, CD70, etc. Incubation of NK cells with IL-18 has been shown to enhance their helping functions [23]. This NK cell "help" function is supported by the reports that in the presence of activated NK cells, antigen-specific CTL may be generated without help from $CD4^{+}$ T cells [24].

NK cell–DC interactions are complex and are important for innate and adaptive immune responses against viral infections. They will be discussed in a later section in this article.

NK cell interactions with other immune cells are important for the induction of effective immune responses. It has been shown that NK cells can regulate adaptive $CD4^{+}$ memory T

TABLE 3. NK Cell Functions

1. Natural cytotoxicity against hazardous (infected or malignant) cells.
2. ADCC against hazardous cells in the presence of antibodies.
3. Immune regulation via secretion of chemokines and cytokines.
4. Immune regulation via physical interaction with other immune cells, e.g., monocyte/macrophages, T and B cells, and DC.
5. Regulate hematopoiesis via secreting GM-CSF.
6. Regulate angiogenesis via interaction with vascular endothelial cells.
7. Uterine NK cells play a role in placentation and pregnancy.
8. Role in contact hypersensitivity.
9. Rejection of bone marrow allografts.

cells. More specifically, adoptive transfer of $CD4^+$ memory T cells specific for myelin oligodendrocyte glycoprotein (MOG; an autoantigen) is able to expand and cause experimental autoimmune encephalitis (EAE; a mouse model of multiple sclerosis) in RAG-2-deficient mice, which lack T and B cells but have functional NK cells. However, NK cells in these mice are inhibited from killing the $CD4^+$ memory T cells, as the latter expresses Qa-1 (the mouse equivalent of human HLA-E). The transferred cells do not cause EAE in Qa-1 lacking mice. NK cells kill MOG-specific $CD4^+$ memory T cells in these mice. These results show that NK cells play an important role in the homeostasis of memory T cells and may also eliminate autoreactive $CD4^+$ memory T cells under appropriate conditions. The results suggest that blocking Qa-1/NKG2A interactions may represent a better clinical strategy to eliminate autoreactive T cells than using anti-CD3 antibodies [25].

In certain mouse models, NK cell activation has been shown to be indispensable for inducing antitumor antibody and CTL responses [17, 26]. In addition to producing IFN-γ, physical interactions between NK cells and other immunocytes are needed for these responses. For example, via CD40/CD40L interactions, NK cells can induce transcription of activation-induced cytidine deaminase and switch recombinations in B cells [27].

NK cells are plastic and may differentiate themselves into cell types that produce predominantly IFN-γ or IL-5. It is not known what causes this polarization in NK cells. It has also been suggested that these differences in cytokine production may result from their different differentiation states. Nevertheless, this polarized production of cytokines from NK cells may be correlated with certain disease conditions. For example, IL-5- and IFN-γ-producing NK cells were shown to correlate with remissions and relapses in multiple sclerosis in humans, respectively [28]. The NK cells producing predominantly IL-5 also expand and play a role in asthma in humans [29].

In a provocative study, NK cells were shown to mediate memory-type responses. In RAG−/− mice, which lack T and B cells, O'Leary et al. [30] demonstrated NK cell-dependent, anamnestic responses to a hapten in mediating contact hypersensitivity. Although the molecular mechanisms behind this NK cell-dependent memory are not clear, the results may have important implications for development of vaccines.

NK cells play an important role in successful pregnancy and reproductive efficiency. The KIR2DL4/HLA-G interactions and consequent secretion of IFN-γ from uterine NK cells are needed for placentation [31, 32].

## NK CELL ACTIVATION

NK cells exist in the body in a semi-activated state and are ready to kill susceptible target cells. However, they may undergo activation in vitro and in vivo, which enhances their functional capabilities. Various stimuli that activate NK cells are listed in **Table 4**. A variety of cytokines activates NK cells and induces their proliferation as well. Activated NK cells have increased expression of activating receptors, adhesion molecules, and intracellular effector molecules, e.g., perforin. They secrete higher amounts of cytokines and have higher cytolytic potential and a reduced threshold to trigger their killing mechanism. Virus-induced cytokines usually result in activation of NK cells of the host. These cytokines include IFN-α/β, IL-2, IL-12, IL-15, IL-18, IL-21, and others [14, 33]. Of these cytokines, IL-15, IL-21, and fms-like tyrosine kinase 3 ligand have been found to be essential for the development, differentiation, and homeostasis of NK cells. IL-15 knockout (KO) mice are deficient in NK cells. Furthermore, NK cells from normal mice undergo apoptosis when transfused into IL-15 KO mice (reviewed in ref. [34]).

TABLE 4. How NK Cells Become Activated in Viral Infections

NK cells may become activated and undergo expansion in a viral infection in several ways:

1. Recognizing viral proteins and/or virus-induced proteins in the host cells via activating receptors.
2. Monitoring the expression of MHC or MHC-like ligands for NKRs.
3. Recognizing viral proteins, DNA, or RNA via so-called pattern recognizing receptors, e.g., TLR, but may require accessory cell help.
4. Virus-induced cytokines from DC, monocyte-macrophages, e.g., IFN-α/β, IL-12, IL-15, IL-18, IL-21, IL-23, etc.
5. By interaction with other cell types, e.g., DC, macrophages.
6. Recognizing genotoxic stress-induced, *de novo*-expressed ligands for the NKG2D receptor.

NK cells also express TLRs. Therefore, they can undergo activation upon binding with certain viral proteins and nucleic acids via these receptors [35, 36]. However, NK cells require accessory cell function (presence of $CD14^+$ monocytes or plasmacytoid DC) for TLR-mediated activation [37–39]. Certain viral proteins may act as ligands for one or more activating NKRs and therefore, may directly activate NK cells; e.g., HA of the influenza virus activates NK cells via NKp46 and NKp44 [40]. NK cells also become activated when they contact a susceptible target cell (e.g., K562, which has reduced expression of MHC class I and/or has increased expression of ligands for certain activating NKRs) and trigger their cytolytic machinery to kill the target cell.

NK cells mainly kill their target cells by releasing cytotoxic molecules (perforin, granzymes, and granulysin), which are normally contained in their granules. These molecules are released within the immune synapse (IS) onto the surface of the target cells. NK cells can also kill target cells by FasL, TRAIL, and TNF-α if the target cells express appropriate receptors [41, 42]. NK-cell mediated lysis is usually determined in microcytotoxicity assays by measuring the release of $^{51}$chromium, lactate dehydrogenase, or perforin in culture supernatants. Individual NK cells mediating the lysis can be recognized and counted by detecting the expression of lysosomal protein lysosome-associated membrane protein-1 (CD107a) on their surface [43, 44].

## REARRANGEMENT OF CYTOSKELETON IN NK CELL-MEDIATED KILLING

As mentioned above, NK cells mainly kill their target cells via releasing cytotoxic granules. To avoid unintended damage to

neighboring cells, NK cells release these effector molecules in a well-defined and restricted area of contact with the target cells (reviewed in ref. [45]). This area of contact between the two cells is called IS. The synapse is an activating one if it is formed between a NK cell and a susceptible target cell. The formation of the synapse is initiated by interaction between different activating receptors and adhesion molecules (e.g., CD2, LFA-1, CD11a, CD11b) on NK cells and their cognate ligands on the target cell. The adhesion molecules aggregate in the form of a ring at the periphery of the synapse (**Fig. 1**). The engagement of integrins causes their activation and results in talin-mediated actin polymerization. The close contact between NK and target cell membranes induces reorientation of the MTOC of the NK cell toward the synapse. The microtubule-associated motor proteins (kinesins) shuttle granules containing cytotoxic mediators toward the center of the synapse. Within the center of the synapse, different activating NKRs interact with their cognate ligands on the target cell. The glycosphingolipid-rich membrane microdomains (lipid rafts) rapidly accumulate in the center of the synapse in a cytoskeleton-dependent manner. The lipid rafts are rich in effector signaling molecules. Their recruitment to the synapse requires activities of several kinases (Src, Syk, and the serine-threonine kinase protein kinase Cε). A ring of F actin surrounds the center of the synapse and prevents spillover of the cytotoxic mediators from the synapse. Within the synapse, NK and target cells interact with each other via membranous protrusions, which end at coated pits on the surface of opposing cells [46]. The Src homology 2 (SH2) domain-containing phosphatase (SHP)-1 is recruited to the periphery of the synapse within 1 min. It limits the activation event to the center of the synapse. Actin polymerization and MTOC reorganization are key events needed to trigger NK cell cytotoxicity (degranulation). The pharmacological agents that inhibit actin polymerization also inhibit NK cell-mediated killing [47]. Another phosphatase, SHIP, is also recruited to the synapse within minutes to terminate the triggering of the NK cell. After discharging its cytotoxic mediators to the membrane of the target cell, the NK cell separates itself and is ready to kill another target cell. A single NK cell can kill several target cells in a sequence one after the other. The inhibitory IS is formed between an NK cell and a resistant target cell. Its formation is prevented at an early stage by inhibitory receptor-recruited phosphatases. The inhibitory receptors cluster in discrete microdomains in the center of the inhibitory synapse and interact with their cognate ligands.

A characteristic feature of the activating IS is exchange of membrane proteins (receptors, ligands, adhesion molecules, etc.) between the interacting cells. For example, NK cells may transfer inhibitory KIR to target cells. The latter cell types may transfer MHC-class I chain-related protein A (MICA) to NK cells, which in turn, may transfer these acquired proteins to other NK cells via homotypic, intercellular contacts [48, 49].

NK cells, like other immune cells, can also communicate with other cells at a distance by forming nanotubules and transfer molecules and $Ca^{++}$ fluxes to them (reviewed in ref. [50]).

## NKRs

NKRs and coreceptors regulate NK cell-mediated killing. They include a variety of molecular structures expressed on the surface of NK cells. They bind specific ligands on target cells. NKRs can be divided into inhibitory and stimulatory types depending on the nature of the signal they send to NK cells

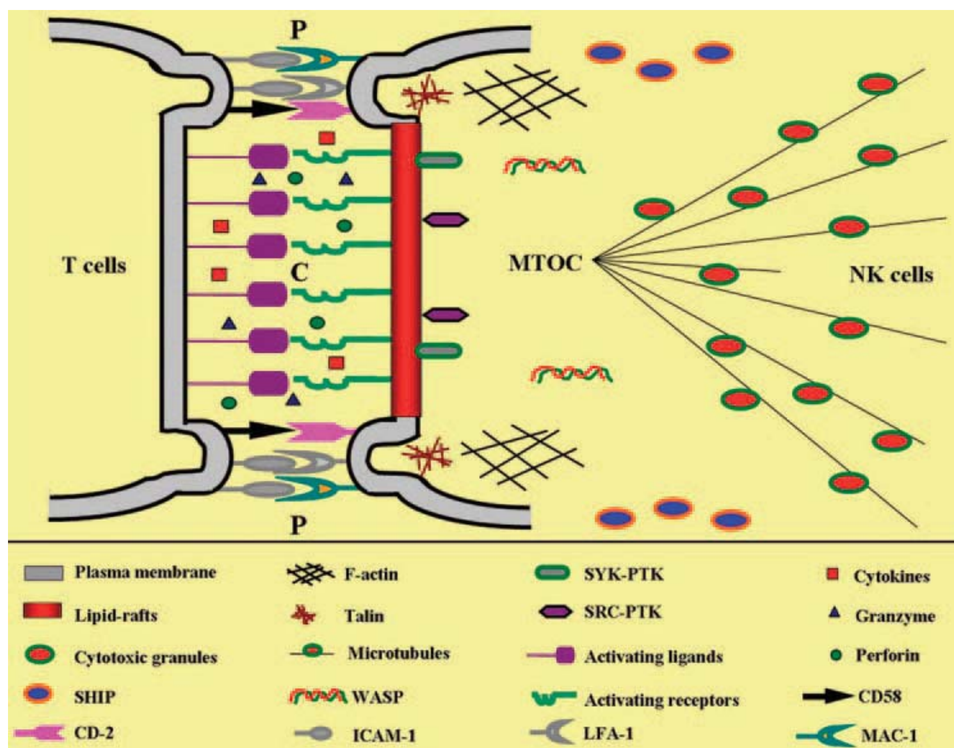


**Fig. 1.** Formation of IS between NK and target cells. Various NKRs interact with their cognate ligands on the target cell in the center of the synapse, which contains lipid rafts and activating NKRs. The adhesion molecules [CD2, LFA-1, membrane-activated complex 1 (MAC-1)] bind to their cognate ligands on the target cell and aggregate in the form of a ring. Activation of integrins leads to talin-mediated actin polymerization. The close contact between NK and target cell membranes induces reorientation of the microtubule-organizing center (MTOC) of the NK cell toward the synapse. The microtubule-associated motor proteins (kinesins) shuttle granules containing cytotoxic mediators toward the center of the synapse. Within the center of the synapse, different activating NKRs interact with their cognate ligands on the target cells. A ring of F actin surrounds the center of the synapse, preventing the spillover of the cytotoxic mediators from the synapse. T and NK indicate target cell and NK cell, respectively. C and P represent center and periphery of the synapse. WASP, Wiskott-Aldrich syndrome protein; PTK, protein tyrosine kinase; SYK, spleen tyrosine kinase; SRC, sarcoma tyrosine kinase.

after binding to their specific ligands. Each NK cell expresses inhibitory and stimulatory NKRs. Target cells may vary from one another with respect to the level of expression of ligands for different NKRs. According to the prevalent wisdom, triggering of an NK cell depends on the balance between inhibitory and stimulatory signals that it receives from the target cell via NKR (see **Fig. 2**). A NK-sensitive target cell usually would express one or more ligands for activating NKR, whereas a NK-resistant target cell would express more ligands for inhibitory receptors and/or may lack ligands for activating NKR. The human NKR can be divided into MHC and non-MHC-binding receptors.

## MHC-binding receptors

They include KIR, NKG2, and Ig-like transcript (ILT) families. They are discussed below.

### *KIR (CD158) family*

The KIR are type I integral membrane glycoproteins that are usually expressed as monomers on the cell surface [51–53]. At present, 14 distinct *KIR* genes and two pseudogenes have been described (see http://www.ebi.ac.uk/ipd/kir for an update on *KIR* genes and alleles). They are located on human chromosome 19q13.4 in a tandem head-to-tail manner in a short, 150-kb region, called leukocyte receptor complex (LRC). *KIR* genes show extensive allelic polymorphism. For example, *KIR2DL1* and *KIR3DL1* genes have at least 14 and 47 alleles, respectively. Their transcripts also undergo alternate splicing, giving rise to distinct receptor variants. It has been estimated that after MHC, KIR is the most polymorphic locus in humans. *KIR* genes are not present in mice, suggesting their recent evolution after divergence of the two species 5 million years ago. They are undergoing rapid evolution in humans under pressure from pathogens, malignancy, and autoimmunity. Two human populations living next to each other and having similar *HLA* genes frequently differ with respect to their *KIR* genes. This observation suggests that the latter genes are evolving faster than the former ones.

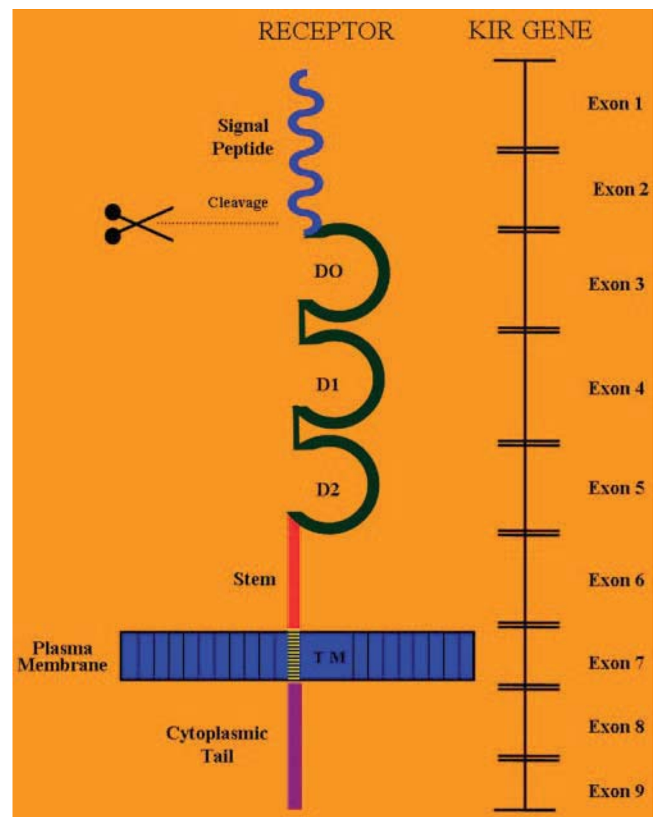


**Fig. 3.** Structure of a typical KIR gene and its encoded receptor. A typical KIR gene comprises nine exons shown here on the right side of the figure. Double horizontal lines in the gene indicate introns. The schematic structure of the encoded receptor is shown on the left. The part of the receptor encoded by each individual exon is also indicated. The scissor in the figure indicates cleavage site for the signal peptide. The letters N and C designate N- and C-terminals of the protein, respectively; not drawn to the scale. D0, -1, -2, Extracellular Ig-like domains; TM, transmembrane region.

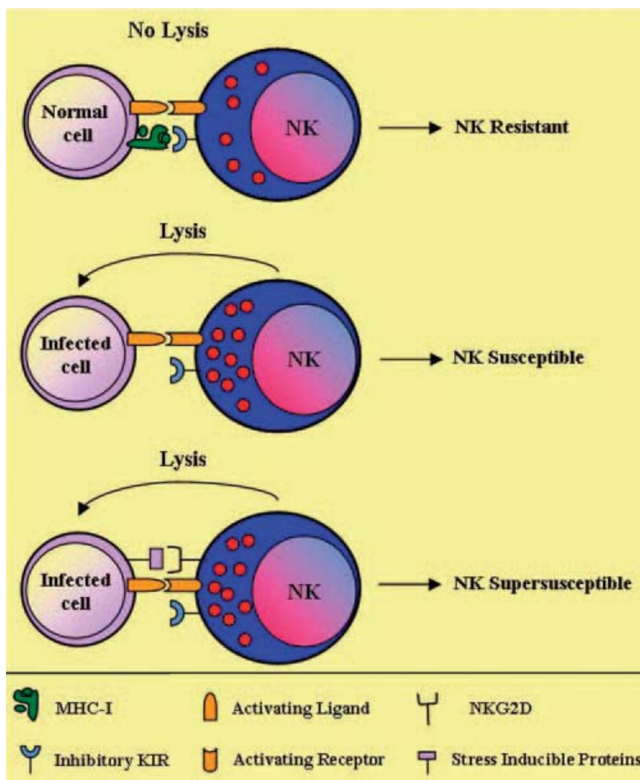


**Fig. 2.** Model for NK cell-mediated killing of virus-infected cells. Normal cells express ligands (usually MHC class I molecules) for inhibitory receptors of NK cells and are resistant to NK cell-mediated killing (top panel). Viral infections may reduce the expression of these inhibitory ligands on the infected cells and make them susceptible to killing by NK cells (middle panel). They may further induce expression of ligands for activating NKRs (e.g., for NKG2D) and make them super-susceptible to the killing (bottom panel).

***KIR structure.*** A typical *KIR* gene contains nine exons as illustrated in **Figure 3**. The exons encode leader sequence (exons 1 and 2), extracellular Ig-like domains (D0, D1, and D2; exons 3–5), stem (exon 6), transmembrane region (exon 7), and cytoplasmic tail (exons 8 and 9) of the KIR. The two-domain KIR lack an extracellular domain (D0 or D1). The ones lacking D0 (KIR2L1, KIR2DL2/3) are called type I KIR, whereas the ones lacking D1 (KIR2DL4 and KIR2DL5; see below) are

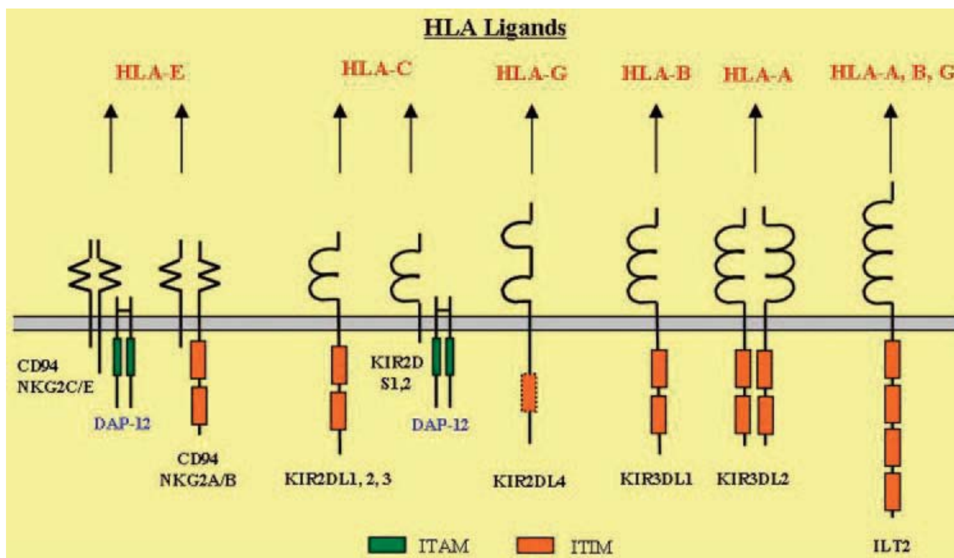


**Fig. 4.** MHC-binding NKRs. The figure shows the schematic structures of main MHC-binding receptors: KIR, ILT-2, and CD94/NKG2. The ligands for the receptors are also shown on the top of each receptor; not drawn to the scale. DAP-12, Dynax activation protein 12.

called type II KIR. The structure of a typical *KIR* gene along with the receptor is shown in Figure 3. The extracellular region of the receptor binds with its ligand and consists of two or three Ig-like domains. The cytoplasmic tail transduces receptor-initiated signals. Depending on the length of the cytoplasmic tail, a KIR could be short-tailed (S) or long-tailed (L). The L forms are usually inhibitory KIR and have two ITIMs (with canonical sequence I/VxYxxL) in their cytoplasmic tails, as depicted in **Figure 4**. Upon binding to their ligands, the tyrosine residues in the ITIMs become phosphorylated and recruit SHP-1 and -2. These phosphatases dephosphorylate several substrates involved in the NK cell activation cascade, e.g., Vav, ζ-associated protein 70 (Zap-70), Syk, phospholipase C (PLC)-γ1, Shc, and SH2 domain-containing leukocyte protein of 76 kDa, and inhibit the NK cell from triggering its cytotylic machinery and secreting cytokines. The receptors with a short (S) cytoplasmic tail are stimulatory. They lack ITIMs but possess a positively charged amino acid (lysine) in their transmembrane regions. Via this amino acid, they associate noncovalently with a dimer of an adaptor protein, killer cell-activating receptor-associated protein (KARAP) or DAP-12 [54]. Each DAP-12 carries ITAMs [with canonical sequence D/Ex(0–2)YxxL/Ix(6–8)YxxL/I] in its cytoplasmic tail. When an activating KIR binds to its ligand, the tyrosine residues in the ITAMs are phosphorylated and recruit various tyrosine kinases, e.g., Syk and Zap-70, and send activating signals to NK cells to kill target cells and secrete cytokines. The signals converge to phosphorylate Vav, which is a guanine nucleotide exchange factor (GEF) and activates the Rho family of small GTPases. Upon activation, the GTPases cause actin polymerization, cytoskeleton rearrangement, and triggerring of degranulation (reviewed in ref. [45]).

The KIR are named, depending on the number of Ig-like domains (2 or 3) present in the extracellular region as well as the length of the cytoplasmic tail (L or S). Each *KIR* gene and its alleles are given different numbers. The KIR expressed in humans, their signaling partners, and ligands are given in **Table 5**.

TABLE 5. Human KIR (CD158) and Their Ligands

| Receptor | Signaling partner | Distribution | Ligand |
|---|---|---|---|
| A. Inhibitory KIR | | | |
| 1. KIR2DL1 (p58.1) | SHP-1, 2 | NK, CTL | HLA-C II |
| 2. KIR2DL2/3 (p58.2) | SHP-1, 2 | NK, CTL | HLA-C I |
| 3. KIR3DL1 (p70; NKB1) | SHP-1, 2 | NK, CTL | HLA-B Bw4 |
| 4. KIR3DL2 (p140) | SHP-1, 2 | NK, CTL | HLA-A3, A11 |
| 5. KIR2DL4[a] | FcεR1γ-chain | NK | HLA-G |
| 6. KIR2DL5 | SHP-2 | NK, CTL | HLA-G? |
| B. Activating KIR | | | |
| 1. KIR2DS1 (p50.1) | DAP-12 | NK | HLA-C II, ? |
| 2. KIR2DS2 (p50.2) | DAP-12 | NK | HLA-C I, ? |
| 3. KIR2DS4 (p50.3) | DAP-12 | NK | HLA-Cw4, ? |
| 4. KIR2DS3, 5 | DAP-12 | NK | ? |
| 5. KIR3DS1 (p70) | DAP-12 | NK | HLA-Bw4-I, ? |

All KIR are expressed clonally on overlapping subsets of NK cells except KIR2DL4, which is expressed on all NK cells. Inhibitory KIR are also expressed on CTL of the effector/memory phenotype. Activating KIR may be expressed on CD4+ T cells in some disease conditions but are rarely expressed on CTL. HLA-Bw4-I designates Bw4 allotypes having isoleucine at position 80. The question mark (?) indicates unknown and/or controversial ligands. [a] Despite having an ITIM-containing, long cytoplasmic tail, it functions as an activating receptor.

***Two-domain KIR and their ligands.*** KIR bind to supertypic public determinants expressed by a subset of closely related HLA or MHC class I antigens. The two-domain receptors (e.g., KIR2DL1, KIR2DL2/3) bind to HLA-C antigens. It is noteworthy that all HLA-C antigens show a dimorphism at position (p)80 in the amino-acid sequences of their α-2 helices: They have asparagine or lysine. The HLA-C with an asparagine at p80 (e.g., HLA-Cw2, -4, -5, -6, -17, and -18) are called group I HLA-C, whereas the ones with a lysine at p80 (e.g., HLA-Cw1, -3, -7, -8, -13, and -14) are called group II HLA-C. The KIR2DL1 binds group II HLA-C, and KIR2DL2 and its allelic variant (KIR2DL3) bind group I HLA-C [53, 55–58] (reviewed in refs. [53, 54]). Thus, each HLA-C allotype is recognized by KIR2DL1 or by KIR2DL2/3. Interestingly, the amino acid at p44 of the protein sequence of a KIR determines its specificity for a given HLA-C group: If it is methionine (as in the case of KIR2DL1), the KIR binds group II HLA-C, and if it is lysine (as in the case of KIR2DL2/3), the KIR binds group I HLA-C [56]. It may be relevant to mention here that these ligand specificities are not absolute: KIR2DL1 and KR2DL2/3 may bind to group I and group II HLA-C ligands, respectively. However, they do so with tenfold lower affinities.

KIR2DL4 (CD158d) is unusual in several respects. First, it binds HLA-G: a nonclassical HLA antigen. Second, it is expressed mainly in endocytic vesicles and binds internalized, soluble HLA-G. Third, the receptor is expressed usually by all human NK cells. Its expression can also be increased by cytokines, e.g., IL-2. Fourthly, the receptor has an ITIM-containing, long cytoplasmic tail (a typical feature of an inhibitory KIR) as well as a charged amino acid (arginine) in its transmembrane region (a feature of an activating KIR). The presence of a charged amino acid in its transmembrane region allows it to associate noncovalently with the signaling adaptor molecule γ chain of the FcεRI. Cross-linking of the receptor with HLA-G-expressing target cells induces secretion of IFN-γ from NK cells but does not trigger cytotoxicity [59–64]. It is noteworthy that HLA-G is expressed in the female reproductive tract, invading placental trophoblasts and thymic epithelial cells. The HLA-G/KIR2DL4 interactions may be important in generating local immune responses against invading pathogens in the female reproductive tract. Uterine NK cells express KIR2DL4, and secretion of IFN-γ from uterine NK cells has been shown to be crucial for vascularization of placenta (reviewed in ref. [65]). KIR2DL4 is expressed at lower levels on uterine NK cells in women undergoing spontaneous, recurrent abortions [32]. Gomez-Lozano et al. [66] have described a multiparous woman lacking *KIR2DL4* in her genome. It is probable that a KIR2DL4-like receptor may have compensated the function of this receptor in this woman.

The *KIR2DL5A* and *KIR2DL5B* were considered as allelic forms of the same gene. However, now, it has become quite obvious that they represent two closely related but distinct genes, which are present in telomeric and centromeric halves of the *KIR* gene cluster, respectively [67]. The KIR haplotypes may have both, none, or one of the two genes. In fact, the *KIR* genes are evolving so rapidly that our conventional ways of distinguishing between alleles and genes are being challenged. *KIR2DL5A* (previously known as *KIR2DL5*001* allele) is expressed, like other clonally expressed *KIR* genes, as a monomer on $CD56^{low}$ NK cells in a variegated manner. It is also expressed on a subset of T cells [68]. Individuals (52–80%) may express this receptor, depending on the population group. *KIR2DL5B* is a hybrid gene that arose from recombination between *KIR2DL5A* and *KIR3DP1* genes. It has three alleles, *KIR2DL5B*002*, **003*, and **004*, of which only **003* is expressed. The *KIR2DL5 A* and *B* genes have 99.58% sequence similarity in their exons. Each KI2DL5 receptor has two ITIMs, of which one is atypical. The receptor has inhibitory function and preferentially recruits SHP-2 [69]. The ligand for KIR2DL5 is not known. However, their extracellular regions are similar to that of KIR2DL4. Therefore, it is believed that KIR2DL5 receptors also bind HLA-G. Interestingly, *KIR2DL5* genes are conserved among primates.

***Three-domain KIR and their ligands.*** Of the three-domain KIR, KIR3DL1 (p70) is expressed as a monomer on the surface of NK cells. It binds to HLA-B and HLA-A allotypes bearing the HLA-Bw4 serospecificity. It is noteworthy that all HLA-B allotypes can be divided into two mutually exclusive Bw4 or Bw6 serospecificities depending on residues 77–83. About one-third of all HLA-B and some HLA-A allotypes are $Bw4^+$. The remaining two-thirds of the HLA-B allotypes is $Bw6^+$. It may be relevant to mention here that HLA-Bw4 allotypes also show dimorphism at p80 of their amino-acid sequence: They may have isoleucine (HLA-Bw4-I) or threonine (HLA-Bw4-T) at this position. It was demonstrated that KIR3DL1 receptors bind the former HLA-Bw4 allotypes with higher affinity [70, 71]. As mentioned above, the *KIR3DL1* gene exists in 47 allelic forms, encoding 41 distinct allotypes, which differ in their ability to bind their MHC ligands. Interestingly, this binding is dependent on the peptide bound to the peptide-binding groove of the MHC class I ligand [72–74]. Some KIR3DL1 allotypes (*001, *002, *008, *015, *009) are expressed at relatively higher levels on the surface of NK cells, and others (*005, *007) are expressed at lower levels. Epidemiological data from Martin et al. [75] suggest that the highly expressed KIR3DL1 allotypes bind HLA-Bw4-I allotypes with high affinity, as their coinheritance gives the highest protection from AIDS in HIV-infected individuals. Furthermore, it also suggests that the lowly expressed KIR3DL1 allotypes bind with HLA-Bw4-T allotypes better than with HLA-Bw4-I ones. This assumption explains the better protection provided by these KIR allotypes, when they are coinherited with HLA-Bw-T as compared with HLA-Bw4-I allotypes. However, these findings need to be tested by direct-binding assays. One KIR3DL1 allotype (*004) is not expressed on the cell surface and remains intracellular. Still, it appears to be of some functional significance [74–76].

Although *KIR3DS1* encodes an activating KIR, it segregates as an allelic variant of *KIR3DL1*. To date, 12 allelic variants have been described, which encode 10 distinct allotypes of KIR3DS1. It is noteworthy that KIR3DS1 and KIR3DL1 show more than 95% sequence homology in their extracellular domains. Genetic epidemiological data strongly suggest HLA-Bw4-I allotypes as ligands for KIR3DS1 [77]. Nevertheless, KIR3DS1 failed to bind HLA-Bw4 tetramers as well as HLA-Bw4 ligands when expressed in EBV-transformed human cells

[78]. It is likely that the receptor binds to HLA-Bw4 ligands when the latter has bound a certain foreign peptide.

KIR3DL2 (NKAT4; p140) is expressed as homodimers on the surface of NK cells. It binds different HLA-A antigens when complexed with certain peptides derived from the viral protein EBV nuclear antigen 1 (reviewed in ref. [53]). The ligands for other KIR are not yet known (see Table 5).

***Affinities of KIR for their ligands.*** It is important to note that KIR differ in their affinities for their MHC ligands. For example, the KIR2DL1 binds with group II HLA-C with higher affinities than does KIR2DL2 for its respective MHC ligands (group I HLA-C). Furthermore, KIR2DL2 has higher affinity for its ligands than its allelic variant KIR2DL3. Similarly different KIR3DL1 allotypes bind to HLA-Bw4 allotypes with different affinities. These differences in affinities of different KIR for their respective MHC ligands are important from the functional point of view as they translate into different levels of inhibition of the NK cells. The differences in affinities are more pronounced between activating and inhibitory KIR.

The S KIR have activating functions. They may represent an allelic variant of an inhibitory *KIR* gene (e.g., *KIR3DS1* is an allelic form of the *KIR3DL1*) or may represent a distinct activating KIR gene (e.g., *KIR2DS4*). It is believed that activating KIR bind the same HLA antigens as do their inhibitory counterparts but with several orders of magnitude lower affinities. In fact, many authors believe that these receptors may bind some unknown ligands expressed by human pathogens, malignant cells, and/or they may bind their cognate MHC ligands that have bound foreign pathogen-derived peptides [52, 53, 79]. It is noteworthy that KIR2DS4 was reported to bind an unknown ligand present on the surface of melanoma cells [80]. The idea is supported further by the fact that certain activating forms of LY49, which are functional homologues of KIR expressed on murine NK cells, bind certain viral proteins. For example, an activating receptor LY49H, which is present on NK cells in C57BL/6 mice, binds a murine CMV (MCMV)-encoded glycoprotein m157 and protects the host from the virus [81]. The virus-susceptible mouse strain 129/SvJ lacks this receptor and instead expresses an inhibitory receptor LY49I for the viral glycoprotein on NK cells. The viral glycoprotein m157 is an MHC class I homologue encoded by MCMV to evade the host's NK cell responses [1]. Another activating receptor LY49P recognizes MHC class I antigens (H-$2D^K$) in mice when bound with a viral peptide [82].

A functional consequence of the different affinities of the inhibitory and activating KIR for their MHC ligands is that under physiological conditions, inhibitory KIR act as dominant-negative regulators for NK cell functions. They are the main receptors that regulate NK cell functions in humans and maintain tolerance of NK cells toward one's own cells.

***KIR-binding and MHC-bound peptides.*** The binding of two-domain KIR to their MHC ligands is sensitive to the nature of the bound peptide. Certain amino-acid side-chains at p7 and p8 of the peptide may interfere with the binding [83]. Otherwise, these KIR do not distinguish between self and nonself peptides bound to their MHC ligands. However, the three-domain KIR are sensitive to the peptide bound to Bw4. Usually, they can bind to their MHC class I ligands complexed with endogenous peptides. This prevents NK cells from killing the body's own cells. The binding of a foreign peptide to their MHC ligands may abrogate their binding with these KIR. This may result in loss of the KIR-imposed inhibition on the NK cell. For example, the binding of a neomycin-derived peptide to HLA-B27 abrogates its recognition by KIR3DL1 [84]. Neomycin-expressing cells are no longer recognized by NK cells expressing this KIR and hence, may be killed. Such interference with KIR recognition may have consequences for the cell's susceptibility to NK cell-mediated killing. In this respect, the three-domain KIR behave as TCRs as far as recognition of HLA molecules is concerned. However, the consequences of the recognition are quite opposite to each other: T cells recognize HLA via TCR and kill target cells, whereas NK cells recognize HLA via KIR3DL1 and spare them from killing. As three-domain KIR may recognize several HLA ligands in association with one or more pathogen-derived peptides, they essentially remain pattern-recognizing molecules, and TCRs recognize only a particular HLA in association with a well-defined, single foreign peptide and are antigen-specific.

#### *CD94/NKG2C killer lectin-like receptor (KLR)-C (NKG2/CD94 family)*

They are also known as the NKG2/CD94 family of receptors. The receptors of this family are type II, C-type, lectin-like integral membrane glycoproteins. As shown in Figure 4, they are expressed on the cell surface as heterodimers with CD94 (NKp43; KLR-D1), which is also a type II, C-type, lectin-like polypeptide. CD94 lacks a cytoplasmic tail and cannot transduce signals. However, it is essential for the cell surface expression of NKG2 receptors. Members of this family as well as other non-KIR human NKR are given in **Table 6**. There are four receptors in the family: A/B (KLR-C1), C (KLR-C2), E/H (KLR-C3), and F (KLR-C4). B and H represent splice variants of A and E genes, respectively [52, 54, 85]. The genes for these receptors are located on human chromosome 12p12.3–p13.2 in a region called NK gene complex (NGC). Of these receptors, CD94/NKG2A has inhibitory function, and it carries two ITIMs in its long cytoplasmic tail. It is expressed in a subset of human NK cells having the $CD56^{high}$ $CD16^{low}$ phenotype. It is also expressed, albeit at lower levels, on the $CD56^{low}$ subset of NK cells. NKG2C has a short cytoplasmic tail, associates noncovalently with a homodimer of DAP-12, and activates NK cells upon binding with its ligands. NKG2E is also considered an activating receptor. It has a charged amino acid (lysine) in its transmembrane region, but it does not associate with DAP-12. The NKG2A and NKG2C are expressed on overlapping subsets of $CD56^+$ NK cells [54]. NKG2F has a truncated extracellular domain comprising only 12 amino acids. It has a charged amino acid (lysine) in the transmembrane region and two ITIMs in its cytoplasmic tail. The protein is retained intracellularly. It does not form heterodimers with CD94 but can complex with DAP-12 [86]. Thus, it may sequester DAP-12 and regulate functional activities of other receptors that use DAP-12 as a signaling partner. Interestingly, DAP-12 has also been implicated in myelination and bone resorption (reviewed in ref. [87]).

The NKG2 receptors bind HLA-E: a nonclassical MHC class Ib molecule [88–90]. HLA-E is expressed in two isoforms

TABLE 6. Non-KIR NKRs

| Receptor | Signaling partner | Distribution | Ligand (function) |
|---|---|---|---|
| 1. NKp46 | FcεR1γ, CD3ζ chain | NK | HA (+) |
| 2. NKp44 | DAP-12 | Activated NK | HA (+) |
| 3. NKp30 | FcεR1γ, CD3ζ chain | NK | ? (+) |
| 4. FcγRIIIa (CD16) | FcεR1γ, CD3ζ chain | NK (s) | IgG, IgE (+) |
| 5. FcγRIIc (CD32) | FcεR1γ, CD3ζ chain | NK (s) | IgG (+) |
| 5. 2B4 (CD244) | SAP, EAT-2, SHIP-1 | NK (s), T (s) | CD48 (+/–) |
| 6. NKG2D | DAP-10 | NK, CTL, Mac | SIP (+) |
| 7. DNAM-1 | Fyn | NK (s) | Nectin-2, PVR (+) |
| 8. TACTILE (CD96) | Fyn | NK (s) | Nectin-2, PVR (+) |
| 9. NKR-P1A | FcεR1γ | NK, NKT, CTL (s) | LLT-1 (+/–) |
| 10. NKG2C/CD94 | DAP-12 | NK and CTL (s) | HLA-E (+) |
| 11. CD94/NKG2A | SHP-1, 2 | NK (s) CTL (s) | HLA-E (–) |
| 12. ILT-2 (CD85j) | SHP-1, 2 | NK (s) B, Mac, DC | HLA-G (–) |
| 13. KLR-G1 | SHP-1, 2 | NK (s), CTL (s) | Cadherens (–) |
| 14. SIGLEC-7 | SHP-1, 2 | NK | Sialic acid (–) |
| 15. NKp80 | ? | NK | AICL (+) |
| 16. FcRL6 | SHP-2 | NK | IgG complexes (–) |
| 17. CD160 (BY55) | PI-3K | NK (s) | HLA-C. G (+) |
| 18. CEACAM-1 (CD66a) | CD16? | NK | CEA and related proteins (+) |
| 19. CD38 | CD16 | NK | CD31 (+) |

AICL, Activation-induced C-type lectin expressed on monocyte/macrophages; CEA, carcinoembryionic antigen; CEACAM-1, CEA-related cell adhesion molecule; EAT-2, Ewing's sarcoma-activated transcript-2; FcRL6, FcR-like protein 6; SAP, signaling lymphocyte-activating molecule (SLAM)-associated protein; SIP, stress-induced proteins [MICA, MICB, UL16-binding protein (ULBP)]; PVR, Poliovirus receptor; DNAM-1, DNAX accessory molecule 1; TACTILE, T cell-activated increased late expression; SIGLEC-7, sialic acid-binding Ig-like lectin 7. The designations (+), (–), and (+/–) indicate that the function is activation, inhibition, and both, respectively. (s) indicates that expression is restricted to a subset of the cell population.

in humans, and both bind equally well to these receptors. HLA-E binds a peptide, VMAPRTVLL, which is present in the signal sequences of many classical MHC class I molecules (HLA-A, -B, -C), and a nonclassical MHC class I molecule HLA-G [89, 91, 92]. Without this peptide, HLA-E does not fold properly and therefore, is not expressed on the cell surface. Thus, NK cells have developed an elegant way to monitor the overall expression of MHC antigens on the surface of target cells by CD94/NKG2-mediated monitoring of HLA-E. Interestingly, human CMV (HCMV) encodes a glycoprotein UL40, which increases the expression of HLA-E on the surface of the virus-infected cells by providing this peptide and hence, increases their resistance to killing by NK cells [93]. HLA-E is normally expressed as well as released by placental trophoblasts, vascular endothelial cells, and peripheral blood cells (monocytes, DC, and T, B, and NK cells). Its expression is increased on human cells by certain cytokines, e.g., IFN-γ, TNF-α, and IL-1β [94]. The expression of HLA-E on human cells may protect them from NKG2A-bearing NK cells, and soluble HLA-E may interfere with this protection.

The KLR family NKRs have nonclassical, lectin-like domains. Therefore, they can bind nonsugar moieties on their ligands [95] (reviewed in ref. [96]). It has been demonstrated in vitro that NKG2A can bind efficiently to HLA-E produced in bacteria.

*ILT (CD85) family*

The family has also been given other names: leukocyte Ig-like receptor (LILR) and macrophage Ig-like receptors. It comprises 13 members. They vary in the number of Ig-like domains present in their extracellular regions and may be inhibitory or activating as in the case of KIR (reviewed in refs. [52, 54]). They are mostly expressed on monocytes, macrophages, DC, and certain subsets of B and T cells. One member of the family ILT-2 (LILRB1; CD85j) is also expressed on a subset of NK cells (**Fig. 5** and Table 6). The receptor has four Ig-like domains in its extracellular region and four ITIMs in its cytoplasmic tail. ILTs bind classical and nonclassical HLA molecules (e.g., HLA-G). ILT-2 preferentially binds HLA-G [97]. It interacts with the α3 domain of the MHC class I molecules and competes with CD8 for binding to the MHC ligand [98]. *ILT* genes are present on chromosome 19 close to the KIR gene cluster.

*CD160 (BY55)*

It is a GPI-anchored, Ig-like molecule expressed on the $CD56^{dim}$ subset of NK cells, γδ TCR-positive T cells, and a subset of $CD8^+$ T lymphocytes. It binds HLA-C, -G and other HLA molecules. NK cell stimulation via CD160 leads to secretion of a unique, proinflammatory profile of cytokines: IFN-γ, TNF-α, and IL-6. A soluble form of the molecule, shed from activated human NK cells, can inhibit functions of $CD160^+$ NK and T cells [99]. CD160-positive NK and CTL are usually implicated in inflammatory conditions.

## Non-MHC-binding receptors

NK cells also express several types of receptors, which recognize different molecular structures (but not MHC antigens) present on the surface of target cells. These receptors are as below.

*NKG2D receptor (KLR-K1; CD314)*

Originally, this receptor was placed in the CD94/NKG2 family. However, it differs from members of this family in many

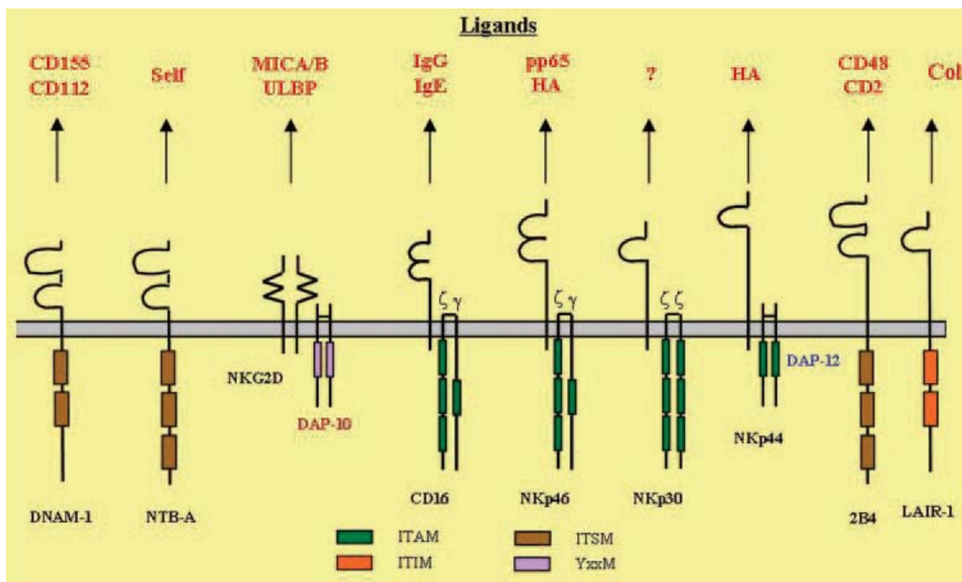


**Fig. 5.** Non-MHC-binding NKRs. The receptors, their ligands, and signaling partners are shown. ITSM, Immunoreceptor tyrosine-based switch motif; Col, collagens. The YxxM motif, when phosphorylated, recruits PI-3K; not drawn to the scale. The question mark (?) indicates that the ligand is unknown. NTB-A, NK-T-B (NK, T-B cell antigen) antigen; LAIR-1, leukocyte-associated Ig-like receptors.

respects. It does not form heterodimers with CD94 (see Fig. 5 and Table 6). It is expressed as homodimers, and each homodimer associates noncovalently with a homodimer of an adaptor protein DAP-10, which is a DAP-12-related protein but does not contain ITAM motifs in its cytoplasmic tails [100]. Instead, DAP-10 carries a YxxM motif, which upon phosphorylation of its tyrosine residues, can recruit the regulatory subunit p85 of PI-3K and growth-factor receptor-bound protein 2. This motif is also present in the cytoplasmic tail of the T cell costimulatory molecule CD28. However, there are important differences between CD28- and NKG2D-transduced signals. The engagement of NKG2D alone, but not of CD28, on NK as well as on T cells allows formation of an IS with target cells [101].

The NKG2D receptors do not recognize and bind HLA-E, as do all members of the CD94/NKG2 family. Instead, they bind MICA, MICB, and the HCMV ULBPs [102]. *MIC* genes are located on human chromosome 6q25 outside the MHC locus. Of the six distinct *MIC* genes, only *MICA* and *MICB* are transcribed. Structurally, MIC proteins resemble MHC class I heavy chain, and each has three (α1, α2, and α3) domains; however, they do not bind antigenic peptides and do not complex with β-2 microglobulin. The *MIC* genes are highly polymorphic. At least 54 *MICA* and 19 *MICB* alleles have been described. The MIC allotypes vary in their ability to bind NKG2D (reviewed in ref. [103]). For example, MICA*01 and 07 allotypes bind strongly, and MICA*016 binds NKG2D weakly. Some allotypes such as MICA*08 and *010 are defective and are not expressed on the cell surface. Thus, an individual's capacity to mediate NKG2D-mediated killing may depend on his/her inherited *MIC* genes. Under physiological conditions, the expression of MICA and MICB is restricted to the basolateral surface of intestinal epithelial cells and fibroblasts. However, they can be induced de novo on different host cells by stress, transformation, and viral infections. More specifically, DNA damage response triggered by stalled DNA replication, genotoxic drugs, irradiation, and hydroxyurea induces expression of MIC and other NKG2D ligands. The response is accompanied by activation of ataxia telangiectasia mutated (ATM) and ATM and Rad-3-related (ATR) kinases. The stimuli, which do not result in the induction of DNA damage response and activation of ATM and ATR (e.g., heat shock, hypoxia, inflammation, TNF-α, or IL-8), do not induce expression of NKG2D ligands [104–107]. MIC proteins may be cleaved via matrix metalloproteinases and shed from the cell surface as soluble proteins, which bind to and down-regulate the expression of NKG2D on NK cells. Tumor cells usually shed these proteins as an immune evasion mechanism [108, 109].

The ULBPs were first discovered by their ability to bind UL-16 of HCMV and appear to have ubiquitous expression at the mRNA level. Five distinct ULBPs (1–5) have been identified. Of these, ULBP3 and -4 do not bind UL16. Structurally, they resemble MIC proteins but lack α-3 domains and could be GPI-anchored (ULBP1, -2, and -3) or have transmembrane regions (ULBP4 and -5; reviewed in refs. [110, 111]). In the mouse, NKG2D recognizes H-60 (a minor histocompatibility antigen), the retinoic acid early inducible protein 1 (Rae-1), and a murine ULBP-like transcript 1 (reviewed in ref. [96]).

NKG2D is an activating NKR. In addition to NK cells, resting and activated human $CD8^+$ T lymphocytes express NKG2D [112]. Both of these cell types can efficiently kill NKG2D ligand-positive target cells. The $CD8^+$ T cells do so without their TCR coengagement [113]. Certain cytokines, e.g., IL-12 and IL-15, can increase expression of NKG2D on these cytotoxic cells, whereas cytokines such as TGF-β and IL-10 can decrease their expression. The cytokine-induced, increased expression of NKG2D on CTL greatly enhances their NK cell-like killing capabilities (reviewed in ref. [111]). NK cells may play an anti-inflammatory role by killing overtly activated macrophages, which start expressing ligands for NKG2D [17]. It is noteworthy that stimulation of macrophages via TLR induces NKG2DL in macrophages [114]. On the other hand, certain cytokines such as IL-1β and TNF-α have been shown to induce MIC and ULBP expression on oligodendrocytes and neurons. NKG2D-mediated killing of oligodendro-

cytes has been shown to play a role in the development of multiple sclerosis in humans [115].

***Natural cytotoxicity receptors (NCRs)***

Three NKRs, NKp46 (CD335), NKp30 (CD337), and NKp44, are called NCRs (Table 6 and Fig. 5). They trigger NK cell-mediated killing and secretion of IFN-γ upon their engagement. NKp46 and NKp30 are expressed on resting and activated NK cells, whereas NKp44 is expressed only on cytokine-activated NK cells [116–118]. NCRs belong to the Ig superfamily (IgSF). They have extracellular Ig domains and associate noncovalently with DAP-12 (NKp44), **γ-ζ** (NKp46), and ζ-ζ chains (NKp30). The ligands for the NCR mostly remain unknown. Two members of the group, NKp46 and NKp44, are known to bind the sialic acid-binding glycoproteins, e.g., HA and HA-neuraminidase, of the influenza and parainfluenza viruses, respectively [40]. α 2,6-Linked sialic acid moieties and the sugar-carrying residue Thr 225 near the membrane proximal region of the receptor play an important role in binding to the ligands [119]. The NCR ligands may also occur on normal hematopoietic cells. For example, NK cells specifically use NKp30 to kill immature DC, suggesting that these cells bear NKp30 ligands. NKp46-positive NK cells protect host from influenza viruses, as the viruses cause lethal infections in NKp46-deficient mice [120].

***NKR-P1 (CD161; KLR-B1)***

The receptors occur as s–s-bonded homodimers on the cell surface. They were first described in mice as NKR-P1C or NK1.1 antigen occurring on NK cells of C57BL/6 mice. In rodents, five distinct genes (*A*, *B*, *C*, *D*, and *F*) have been described. They encode activating receptors except for *B* and *D* genes, which encode inhibitory versions of the receptor. The humans have *A* gene. Its protein (CD161) is expressed on NK cells, NKT cells, and a fraction of $CD8^+$ T cells. The human CD161 binds LLT-1 and transmits activating signals via the γ-chain of the FcεRI. LLT-1 is usually expressed on monocytes and T, B, and NK cells. IL-2 can induce its expression on NK cells. The receptor differentially regulates NK and T cell functions: It increases TCR-mediated IFN-γ production in T cells but inhibits cytotoxicity and IFN-γ production in NK cells [121]. Another study has shown that the receptor cross-linking induces IFN-γ production but not cytotoxicity in human NK cells [122]. Thus, the receptor may perform dual functions. The occurrence of the receptor and its ligand on NK and T cells suggests that the receptor may interact with its ligand in cis.

***SLAM-related receptors (SRRs)***

The SLAM (CD150) is expressed in T cells and transmits its signals via an adaptor protein called SLAM-associated protein (SAP; or SH2D1A). The SRRs include 2B4 (CD244), NTB-A (Ly108), and CD2-like receptor on activated cytoxic cells (CRACC; CD139). These receptors are related to SLAM, as they all use similar signaling molecules, SAP, or related molecules (see Table 6). The genes for SRR are located on human chromosome1q22. They are expressed on NK cells, monocytes, basophils, γδ TCR-bearing T cells, and $CD8^+$ T cells of the effector/memory phenotype (Fig. 5). The receptors bear so-called ITSM (TIYxxV/I) in their cytoplasmic tails. These receptors transmit activating signals via SAP, which has a motif in its SH2 domain centered on Arg 78. This motif binds the SH3 domain of the src family kinase Fyn (reviewed in ref. [123]). SAP-Fyn-mediated signaling is important for TH2-type cytokine responses. Interestingly, SAP KO mice lack NKT cells [124]. A distal tyrosine-based motif (TVYxxV/I) in the cytoplasmic tail of the receptor can recruit SHIP, which can dephosphorylate phosphatidyl 3,4,5-triphosphate to phosphatidyl 3,4-biphosphate. Another SAP-like adaptor protein, EAT-2 or SH2D1B, can replace SAP and associates with SRRs. However, EAT-2 cannot recruit Fyn. In the absence of SAP, 2B4 may act as an inhibitory receptor. As a result of this ability of 2B4 to act as an inhibitory as well as an activating receptor, it is recognized as a receptor with dual functionality. Early in ontogeny when NK cells become cytolytic but still have not yet expressed KIR and NKG2A receptors, they do not express SAP. 2B4 acts as an inhibitory receptor and prevents autoaggression from these developing NK cells. It has been demonstrated that EAT-2 and SAP bind 2B4 in resting and activated NK cells, respectively. The ligand for 2B4 is CD48, which is widely expressed on human cells except plasmacytoid DC. Interestingly, 2B4 in mice mainly acts as an inhibitory receptor. 2B4 KO mice show increased cytotoxicity against CD48-expressing target cells. The mouse NK cells also express a EAT-2-related transducer, which like EAT-2, cannot recruit Fyn. The gene for SAP is located on the X chromosome in humans. It is noteworthy that genetic defects in SAP can cause X-linked lymphoproliferative disease and fatal EBV infections in humans [125, 126]. NTB-A, like 2B4, may bind SAP and EAT-2. The two adapters seem to control cytokine production and cytotoxicity in NK cells, respectively [127]. In resting NK cells, CRACC binds EAT-2 but dissociates from it upon activation and recruits PI-3K and PLC-γ. NK cells may interact with macrophages, CTL, and other cells via 2B4/CD48 interactions.

***KLR-G1 or mast cell function-associated antigen***

It is a type II lectin-like inhibitory receptor expressed as an s–s-bonded dimer on the surface of mast cells. It is also expressed on antigen-specific CTL of the effector/memory phenotype, a subset of $CD56^{dim}$ NK cells, and certain $CD4^+$ T cells in humans as well as in mice [128]. Its cross-linking inhibits cytokine secretion and cytotoxicity but not proliferation. It inhibits IgE-mediated activation of mast cells. The receptors bind ubiquitously expressed endothelial (E), neural, and retinal cadherins or junction proteins [129]. Interestingly, E-cadherins are lost in epithelial tumors undergoing metastasis.

***FcRL***

They make a growing family of molecules with homology to FcγRI. All FcRL are mainly expressed on B cells; however, one member of the family, FcRL6, is expressed on the surface of NK cells as well as on a subset of $CD8^+$ T cells of the effector-memory phenotypes [130]. Its novel cytoplasmic, cysteine-rich motif can recruit SHP-2 and inhibit cellular functions.

TABLE 7. Human NK Cell Coreceptors

| Name | Ligand | Expression | Function |
|---|---|---|---|
| CD2 (LFA-2) | D58 (LFA-3)<br>CD48 (weakly) | All NK | Costimulation, adhesion |
| LFA-1 (CD11a/CD18) | CD54 (ICAM-1–5) | All NK | Costimulation, adhesion<br>Cytoskeleton rearrangement |
| CD8 | MHC class I | Subset | Costimulation, adhesion |
| CD69 | unknown | Activated NK | Costimulation |
| CD56 (N-CAM) | self | Subset | Homotypic adhesion |
| CD59 | C8, C9 | All NK | Adhesion, costimulation |
| CD57 | unknown | Subset | Marker of senescence |
| CD28 | B-7 | Fetal NK | Costimulation |
| CD27 | CD70 | Subset | Costimulation |
| CD44 | Hyaluronic acid | Activated NK | Costimulation, adhesion |
| VLA-4 | Fibronectin, VCAM-1 MAdCAM-1 | Subset | Adhesion, diapedesis |
| VLA-5 | Fibronectin | Subset | Adhesion, diapedesis |

N-CAM, Neural cell adhesion molecule; VCAM, vascular cell adhesion molecule; MAdCAM, mucosal adressin cell adhesion molecule.

#### *NKp80 (KLR-F1)*

It is a type II lectin-like molecule expressed as s–s-bonded homodimers on the surface of NK and $CD3^+CD56^+$ T cells. The cytoplasmic tail has two E/KxYxxL/T tyrosine-based motifs. It is expressed on $NCR^{dull}$ and $NCR^{bright}$ NK cells. The receptor binds AICL, which is a myeloid-specific activating receptor [131]. Its gene is located in the NGC close to that of CD69.

#### *DNAM-1 (CD226)*

The receptor belongs to the IgSF. It is expressed on the surface of NK, T, and a subset of B cells in physical association with LFA-1. Its gene is located on human chromosome 18q22.3 (Fig. 5). Its ligands include PVR (CD155), Nectin-2 (CD112), and nectin-like molecules, which are widely expressed on a variety of cells, e.g., endothelial, epithelial, and neuronal cells and fibroblasts [113]. The receptor is implicated in transendothelial migration, diapedesis, costimulation, and adhesion. A receptor closely related to DNAM-1 is CD96 (TACTILE). It bears 20% homology with DNAM-1 and also binds to nectins and nectin-like molecules.

#### *Four Ig-like B7 homologues (4IgB7H or B7H)*

These receptors belong to the B7 family and are expressed on NK and T cells. They may activate or inhibit the cell. One member of the family, B7-H1 [also called programmed death (PD) ligand-1], is expressed on NK cells. Its expression is increased by certain chemokines (e.g., CXCL-9, -10, and -11) on NK cells [132]. The receptor is also expressed and/or can be induced on several cell types in the body. The receptor interacts with its ligand PD-1 and causes premature activation of naïve T cells and inhibition/apoptosis of antigen-specific effector cells. It may be relevant to mention here that increased expression of PD-1 on HIV-specific T cells has been implicated in immunodeficiency in HIV-infected persons [133].

#### *SIGLEC-7 [p75; adhesion receptor molecule-1; (AIRM-1); CD328]*

SIGLECs are a family of sialic acid-binding Ig-like lectins, which belong to the superfamily of sialoadhesion proteins. Of these molecules, SIGLEC-7 (or AIRM-1) is expressed on the surface of human NK cells. Two other molecules, SIGLEC-9 (CD329) and SIGLEC-3 (CD33), are expressed relatively weakly on human NK cells. The SIGLECs transduce inhibitory signals upon binding with sialic acid moieties.

#### *CEACAM-1 (CD66a)*

It binds CEA and CEA-related antigens, which are expressed on tumor cells. The binding inhibits NK cell-mediated functions [134]. One of its ligands is PECAM-1 (CD31), which is expressed on vacscular endothelial cells as well as on NK cells. Cross-linking CD31 on NK cells activates LFA-1 [135].

#### *LAIR*

Two members of the family, LAIR-1 (CD305) and LAIR–2 (CD306), have been described [52, 54]. They are ubiquitously expressed on all leukocytes including NK cells. LAIR-1 has one Ig-like domain in its extracellular region and one ITIM in its cytoplasmic tail. The receptors bind collagens, which are abundantly expressed proteins in the body. Collagens are not present in blood, so leukocytes are only exposed to the LAIR ligands when they extravasate blood and enter tissues.

## NK CELL CORECEPTORS

NK cells express several coreceptors, which bind to their cognate ligands on target cells and send costimulatory signals. These signals add to the overall strength of the activating signal. Adhesion molecules, which include integrins, selectins, and Ig-like molecules, usually act as coreceptors for NK cells (listed in **Table 7**). The distinction between NKRs and coreceptors is not very clear. Some molecules considered as coreceptors (e.g., LFA-1) may trigger NK cell-mediated lysis under appropriate conditions and hence, may qualify as activating receptors.

Integrins are heterodimers of α and β chains and may be of β1 and β2 types. LFA-1 is a β2 integrin ($\alpha_L$:β2; CD11a/CD18), which is expressed by NK cells and other leukocytes. It is involved in essential early steps in NK cell-mediated killing [136]. It mediates contact and adhesion of NK cells with

the target cell as well as polarization of cytoskeleton (actin and microtubules) and granules in NK cells toward the target cell [44]. It is noteworthy that certain chemokines and cytokines (such as CCL-5, IL-2, and IL-15) as well as inside-out signaling from certain activating receptors (such as CD31) can induce conformational changes in LFA-1. These changes enhance affinity and avidity of the integrin for its ligands. Other β2 integrins expressed by NK cells include type III complement receptor (CR3; Mac-1; $\alpha_M$:β2; CD11b/CD18) and CR4 ($\alpha_X$:β2; CD11c/CD18). The β1 integrins expressed by NK cells include VLA-4 (α4β1; CD49d/CD29) and VLA-5 (α5β1; CD49e/CD29). They were named so because of their late expression in the course of T cell activation. Their ligands are indicated in Table 7. NK cells use these molecules to interact with vascular endothelial cells. These interactions are important for NK cell extravasation and also regulate angiogenesis. The costimulation of NK cells via integrins usually facilitates their cytotoxicity and cytokine secretion. However, coligation of α4:β1 has also been implicated in the inhibition of CD16-mediated killing of NK cells [137].

CD56 is an isoform of the N-CAM and is involved in homotypic cell adhesions. It is an IgSF member. The expression of CD56 on the surface of $CD8^+$ T lymphocytes coincides with their acquisition of cytolytic potential [138]. Twenty percent to 30% human (but not murine) NK cells express CD8 on their surface. This coreceptor stabilizes interaction of NK cells with target cells by binding with MHC [54]. CD69 is an early marker of NK cell activation. Its expression correlates with the cytotoxic potential of NK cells. CD25 represents the IL-2Rα chain. Its expression correlates with the proliferative capacity of NK cells in response to picomolar concentrations of this cytokine. CD27 is a member of the TNFR family. It binds transferin receptor (CD70) on target cells. CD44 binds hyaluronic acid on matrix proteins and facilitates LFA-1-mediated adhesion. CD59 is a GPI-anchored membrane glycoprotein, which physically interacts with NKp30 and NKp46 on the surface of NK cells. It is a complement regulatory protein and protects cells from death as a result of complement activation. It binds to the complement proteins C8a and C9. As a result of its physical association with the NCR, it transduces positive signals upon binding to its ligands and thus, acts as a coreceptor for NK cells.

Finally, resting fetal NK cells but not adult human NK cells express CD28, CD80 (B7.1), and CD86 (B7.2) as coreceptors.

## DIVISION OF LABOR AMONG NKRs

It has been demonstrated that no activating NKR alone is able to kill insect (Drosophila) cells expressing its cognate ligands [44]. The engagement of LFA-1 by its ligands expressed on insect cells causes contact and adhesion as well as polarization of NK cell granules and cytoskeleton. Nevertheless, it does not result in degranulation of NK cells. On the other hand, the engagement of CD16 by human IgG expressed on insect cells causes contact and degranulation of NK cells but not polarization of their cytoskeleton. However, a simultaneous engagement of CD16 and LFA-1 results in contact, adhesion, polarization, and degranulation of NK cells resulting in secretion of cytokines and killing of the target cells. Thus, activating signals emanating from these two distinct receptors complement each other in NK cell-mediated killing of target cells. It has been demonstrated that cross-linking of CD2, 2B4, or NKG2D alone by specific mAb results in minor $Ca^{++}$ fluxes in resting human NK cells [44, 137]. These data suggest that there exists a certain degree of division of labor among NK cell-activating receptors. It is noteworthy that different activating receptors and coreceptos may differ from each other in their signaling pathways. Two activating receptors with disparate signaling pathways may complement each other and trigger NK cell-mediated killing. On the other hand, a simultaneous engagement of two or more receptors with similar signaling pathways (e.g., use of ITAMs) may not result in triggering NK cell funtions. They may simply show functional redundancy and not complementarity. Therefore, a combination of at least two activating receptors with disparate signaling pathways must be coengaged by target cells to provoke NK cell-mediated killing. This implies that to be killed by a NK cell, it is compulsory for the target cell to express cognate ligands for at least two distinct activating NKRs. This provides a safety mechanism against autoaggression by NK cells. The reductionist approach of analyzing functions of activating NKRs in insect cells has challenged traditional concepts of NKRs and coreceptors. In the light of these observations, a reappraisal of the role of various NKRs in the process of NK cell-mediated killing is needed.

## REGULATION OF NK CELL FUNCTIONS BY NKRs

NK cell-mediated killing of target cells is tightly controlled by inhibitory and activating receptors. This control ensures that NK cells do not kill normal, healthy cells of the body and cause tissue destruction (autoimmunity). As mentioned above, KIR and NKG2A are the main NKRs that inhibit NK cell-mediated killing. These receptors bind MHC class I antigens. Therefore, the body's healthy cells, which express normal levels of these self-antigens, are spared from NK cells. The existence of MHC class I antigen-specific inhibitory receptors was in fact predicted by the "missing-self hypothesis" proposed by Kärre's group in the mid-1980s [139]. These workers observed that NK cells preferentially killed tumor cells, which lacked or have reduced expression of MHC class I antigens. They also observed that MHC-positive cells were resistant to killing by NK cells (reviewed in ref. [139]). Their hypothesis was based on these observations. It posited that NK cells sense the expression of MHC class I antigens on the surface of target cells and kill those cells that lack these antigens. The discovery of MHC class I-binding inhibitory receptors (e.g., KIR and LY49 on human and mouse NK cells, respectively) proved validity of their hypothesis. However, NK cells have also been found to express a multitude of activating receptors and coreceptors, which bind to various MHC and non-MHC ligands expressed on the surface of target cells. The discovery of these receptors was a surprise, as their existence was not predicted by the missing-self hypothesis.

The current model of NK cell function is based on the engagement of its inhibitory and activating receptors by their cognate ligands expressed on the surface of target cells. When an NK cell comes in contact with a target cell, inhibitory and activating receptors may bind with their cognate ligands expressed on the surface of the target cell. The balance between inhibitory and activating stimuli received by the NK cell determines whether it will kill or spare the target cell (Fig. 2). Under physiological conditions, inhibitory signals usually remain dominant over activating ones. If for some reason, MHC class I expression is reduced on body cells, it may turn the balance of NK cell-received signals in favor of activating ones (as a result of loss of MHC-mediated inhibition of NK cells) and may make these cells susceptible to NK cell-mediated killing. Viral infections and malignancy may cause a reduction in the expression of MHC class I antigens on the surface of infected cells. That may explain, at least in part, why NK cells can kill virus-infected and cancer cells. However, it may be noted that a MHC-deficient cell is only killed if it expresses ligands for one or more activating NKRs. Furthermore, down-regulation of MHC expression by a target cell is not a prerequisite: It may become killed despite expressing normal levels of MHC antigens if it increases the expression of ligands for one or more activating receptors. Consequently, if target cells express NKG2D ligands de novo, the engagement of KIR may not be able to inhibit NK cell-mediated killing via NKG2D. If target cells express de novo NKG2D ligands and/or increase the expression of ligands for other NK cell-activating receptors as well as down-regulate the expression of MHC antigens, they would become super-susceptible to NK cell-mediated killing. The ligands for the activating NKG2D receptor may be induced on the body cells by viral infections, malignancy, or other stimuli causing genotoxic stress. Thus, NK cells sense not only the "missing or altered self" but also "induced self" to detect hazardous cells and kill them. It is noteworthy that different NK cell clones may vary in their ability to kill a given target cell. This ability depends on the repertoire of its activating and inhibitory receptors as well as its repertoire of signaling and effector molecules (perforin, FasL, etc).

## EXPRESSION OF NKRs

The expression of the main NKR genes, KIR, is controlled at the transcriptional level. Once expressed, the expression remains fairly stable. KIR genes are expressed clonally on overlapping subsets of NK cells in a cumulative and stochastic manner, independently of each other. A developing NK cell keeps on expressing its KIR genes at random one after another until it has acquired an overall level of inhibition that is just sufficient to make the cell tolerant to autologous cells. An individual NK cell does not express all KIR genes present in its genome. It expresses only a subset of these genes. This mode of expression makes NK cells of an individual heterogeneous and variegated with respect to the expression of KIR (reviewed in ref. [140]). All clonally expressed *KIR* genes have more or less similar upstream regulatory regions (reviewed in ref. [141]). These regions are characterized by the presence of at least two promoters: a proximal (5′ to ATG codon) and a distal (located 891–1347 bases upstream of the ATG) promoter. The proximal promoter is composite and consists of overlapping and bidirectional promoters, which work in opposite directions. It can produce sense and antisense transcripts. The sense transcripts from the promoter result in splicing and expression of the KIR protein. The antisense transcripts from the proximal promoter join sense transcripts from the distal promoter, make dsRNA, and silence transcription through RNase III-type nucleases (which cleave dsRNA into small, uniform, 21 bp segments) and RNA-induced silencing kinase (reviewed in ref. [142]). The ratio between forward and reverse transcriptional activities of the proximal promoter of a KIR gene determines whether the gene is expressed in an NK cell. The promoter activities involve cell-specific and nonspecific transcription factors. The bidirectional proximal promoters act as probabilistic binary switches for turning on or off transcription of individual KIR genes (reviewed in refs. [141, 143, 144]). The direction of the transcription, once selected, remains fixed for the life of the cell. The presence of reverse transcripts for a given KIR gene from its bidirectional promoter represents lack of expression of the gene. The KIR genes, not expressed in NK cells, are maintained silent, mainly by DNA methylation of their promoter regions. The transcription start sites in KIR genes are surrounded by CpG islands, which are always methylated in nonexpressed genes. Consequently, treatment of NK cells with demethylating agents, e.g., 5-aza-2 deoxycytidine, causes expression of all silenced KIR genes. It was demonstrated that single base-pair mutations in the acute myeloid leukemia-binding sites in the promoter regions of two *KIR2DL5* alleles (*KIR2DL5.2* and *KIR2DL5.4*) result in their transcriptional silence and nonexpression as a protein [145]. It is noteworthy that the KIR2DL4 promoter is quite different from those present in other clonally expressed KIR genes and is not controlled by probabilistic transcriptional switches. This explains why this KIR gene is expressed ubiquitously.

A consequence of the stochastic expression of KIR genes is that each NK cell of an individual has a unique repertoire of expressed KIR. On the average, three to four *KIR* genes (inhibitory and activating ones) are expressed in an individual NK cell [146–148]. At least one of these receptors binds a self-MHC class I ligand and induces tolerance to self. The KIR genotype of an individual determines the repertoire of KIR expressed on his/her NK cells. The HLA genotype of the person affects this repertoire in a subtle way. The percent of expression of a KIR on NK cells of an individual is slightly increased if he/she also expresses an HLA ligand for that KIR [149]. A higher number of copies of an individual KIR allele also enhance its frequency of expression on NK cells. Furthermore, the number of other inhibitory KIR-HLA ligand pairs expressed in an individual also affects the expression of a given KIR on his/her NK cells; the higher the number, the lower the frequency. Therefore, it is not surprising that KIR haplotype identical sibling pairs with different MHC class I haplotypes have significant differences in the frequencies of expression of different KIR genes on their peripheral blood NK cells [150].

KIR and NKG2/CD94 receptors are usually expressed on mutually exclusive subsets of NK cells and complement each other (Table 2). In the course of NK cell development and differentiation, NKG2/CD94 receptors are expressed earlier.

Later in development, these receptors are replaced by KIR. However, a small proportion of NK cells continues to express NKG2/CD94 receptors and does not express KIR. In blood, $CD56^{high}CD16^{low}$ NK cells usually express NKG2/CD94 receptors.

Although an individual NK cell may express three to four KIR genes, rarely does more than one of these receptors bind to a self-MHC antigen. Consequently, each individual NK cell can sense and respond to changes in individual MHC antigens on autologous cells. It would not have to wait until there is a global decrease in the expression of all MHC antigens. A distinct advantage of the clonal and variegated pattern of expression of KIR on NK cells is that different NK cells can sense different MHC class I antigens on target cells. NK cells in an individual may express certain KIR, which may not bind to any HLA antigen expressed by him/her. The individual may not have inherited HLA ligand genes for the receptor. For example, an individual may have a *KIR2DL1* gene but may lack group II HLA-C genes, which encode its ligands. This may happen, as KIR and MHC class I genes are located on two separate chromosomes (12 and 6, respectively) and are assorted independently of each other.

The expression of KIR genes on NK cells usually remains stable and is least affected by cytokines. IL-21 plays a role in the induction of these receptors on developing NK cells from $CD34^+$ progenitor cells in in vitro cultures [151]. The cytokine, however, does not affect KIR gene expression in mature NK cells. In contrast to KIR, cytokines may regulate expression of other NKRs. For example, IL-15, IL-10, and TGF-β1 were shown to induce expression of CD94/NK2GA on developing NK cells as well as on the TCR-stimulated $CD8^+$ T cells. TGF-β1 also reduces expression of NKp30 and NKG2D on NK cells. IL-21 increases the expression of NCR and 2B4 on NK cells. The cytokine, however, decreases expression of NKG2D on NK cells as well as on CTL. Glucocorticoids also decrease NCR expression on NK cells [152–156]. These studies show that changes in cytokine production, which usually accompany viral infections and malignancy, may cause changes in the expression of different receptors on NK cells as well as on T cells. An altered expression of NKR has important implications for the functional activity of NK cells; it may lead to the emergence of autoimmune NK cell clones if inhibitory receptors on NK cells are reduced, and/or activating receptors are overexpressed. NK cells may also become immunodeficient if inhibitory receptors to MHC antigens are overexpressed on them. It has been demonstrated in the mouse model in vitro and in vivo that blocking NK cell inhibitory receptors by small molecular weight inhibitors or by receptor-specific antibodies increases NK cell activity against tumors and results in their regression [157]. The autoimmune cells may kill normal, autologous cells, whereas the immunodeficient ones may not be able to kill otherwise susceptible malignant or virus-infected cells. A dysregulated in vivo expression of KIR genes has been documented to cause immune deficiency in humans [120]. The authors described the case of a person who expressed KIR2DL1 on all of his NK cells. He was immunodeficient and suffered from repeated viral and bacterial infections.

## ROLE OF INHIBITORY RECEPTORS IN NK CELL "EDUCATION"

It was proposed that each NK cell in an individual expresses at least one inhibitory receptor (KIR or NKG2/CD94) capable of binding to a self-MHC antigen. This ensures that NK cells do not kill normal, autologous cells. However, it has been demonstrated that some NK cells may not express any inhibitory receptors (KIR or NKG2/CD94) specific to a self-MHC antigen. These cells do not kill autologous target cells and are hyporesponsive [158]. According to one school of thought, these cells have not been "licensed" to kill and therefore, are tolerant of autologous target cells. According to the "licensing" hypothesis, NK cells must express inhibitory receptors specific for self-MHC class I antigens for acquiring killing capacity [159, 160]. This hypothesis implies that these NK cells are somehow immature. Another group of researchers thinks that these NK cells are fully mature and capable of killing but are "disarmed" to kill autologous cells. They argue that as a result of persistent activation of these cells, their signaling pathways become attenuated [161]. The disarmed NK cells express all of the phenotypic markers of fully differentiated, mature NK cells. Upon activation with pharmacological activators, e.g., ionomycin, they can release cytokines such as mature NK cells. In conformity with the "disarming" hypothesis, NK cells from Rae-1 transgenic mice are also hyporesponsive [162]. Furthermore, NK cells are chronically activated in transporter associated with antigen processing 2-deficient humans but are hyporesponsive and tolerant to self. This self-tolerance could be ascribed at least partially to their reduced expression of NKG2D, ζ-chain, and NKp46 [10].

## KIR HAPLOTYPES

As mentioned above, all *KIR* genes are tightly packed in a head-to-tail manner in the LRC region (reviewed in refs. [163, 164]). Each KIR gene is ~2.0 kb apart. The order of the genes in the LRC region has been deduced from sequencing of the KIR haplotypes as well from segregation analyses. KIR haplotypes vary in humans with respect to the number of activating and inhibitory genes as well as to their allelic forms. Because of these variations, a large number of KIR haplotypes have been identified. These haplotypes may be classified into two broad types: A and B. The type A haplotypes usually contain five inhibitory KIR genes. They also contain one S or activating KIR gene (*KIR2DS4*). This activating KIR, however, is frequently mutated and encodes a nonfunctional receptor as a result of the presence of a 22-bp deletion in exon 5 of the gene. About 80% of the Caucasians have this deletion [165]. Therefore, type A haplotypes usually do not express a functional, activating KIR. They have an inherent tendency to strongly inhibit NK cells. Type B haplotypes are more diverse and may contain more (up to 14) KIR genes, which may include as many as five activating *KIR* (*KIR2DS1*,- *2*, and *-3*, *KIR2DS5*, and *KIR3DS1* but not *KIR2DS4*). It is noteworthy that all human KIR haplotypes contain *KIR2DL4*, which acts as an activating receptor despite having a long cytoplasmic tail. **Figure 6**

**Fig. 6.** Schematic representation of KIR haplotypes in humans. A haplotypes contain five inhibitory *KIR* and only one activating *KIR* (*2DS4*), which is frequently mutated. B haplotypes differ from A in having many activating *KIR* genes. The framework genes (*3DL3*, *3DP1*, *2DL4*, and *3DL2*; in violet color) are present in each haplotype. The figure shows KIR genes present in the centromeric and telomeric halves of the frequently found A and B haplotypes above and below the framework genes, respectively. Each box in the figure represents a *KIR* gene. *3DP1* is a pseudogene.

shows the prevalent KIR gene arrangements present in the most common A and B types of KIR haplotypes. Four *KIR* genes *KIR3DL3*, *KIR3DP1*, *KIR2D4*, and *KIR3DL2* are present in all KIR haplotypes. Of these, *KIR3DP1* is a pseudogene. These genes have been termed the "framework genes" [163, 166]. The frequencies of the two haplotypes vary significantly in different human populations. For example, ~60% Japanese, 30% Caucasians, and 2% Australian Aborigines are homozygous for A haplotypes [166, 167]. It has been demonstrated that overall, 12% humans have predominantly inhibitory KIR genotypes (more inhibitory receptors than activating ones), and 36% have predominantly activating ones (more activating than inhibitory receptors). The rest (52%) have neutral KIR genotypes (equal number of activating and inhibitory KIR genotypes). Furthermore, only less than 1% of unrelated humans has the same KIR genes [168]. In a small percentage of people, KIR may be truncated or unusually elongated. These invividuals may lack or may have duplicated copies of certain KIR genes. The KIR genotype of an individual greatly affects his/her NK cell responses to viral infections.

## KIR-HLA EPISTATIC INTERACTIONS AND RESISTANCE TO VIRAL INFECTIONS

The KIR bind specific HLA antigens and regulate NK cells functions. The two gene families are located on two different chromosomes in humans. Therefore, they segregate independently of each other in meiosis and may result in unique *KIR/HLA* genotype combinations in humans. These unique *KIR/HLA* combinations translate into unique interactions and control over NK cell functions in humans. The degree of inhibition mediated by interactions between coinherited KIR and MHC class I gene products in an individual determines the activation threshold for his/her NK cells. The differences in the inhibition of NK cells result from different affinities of different KIR for their cognate MHC ligands. Furthermore, individuals may vary in the number of KIR/MHC inhibitory pairs as well as in the number of inherited, activating KIR genes [163, 168]. Apart from playing a direct role in killing virus-infected cells, NK cells effectively regulate several aspects of virus-specific, adaptive immune responses. Moreover, KIR (especially the inhibitory ones) are also expressed on virus-specific $CD8^+$ T cells, and NKR/MHC interactions have been documented to control the effector function of these cells. For this reason, the effects of the KIR/MHC combinations may go beyond NK cells and determine the overall immune efficiency of the individual. For example, if a person X expresses KIR and MHC antigens that interact strongly with each other, he/she would be relatively immunodeficient as compared with person Y, whose KIR and MHC antigens interact relatively weakly with each other. From the point of view of a viral infection, the X may be at a relative disadvantage to mount an effective antiviral immune response and clear the infection as compared with the Y. However, it has also been argued that X individuals may be better than Y individuals in dealing with viral infections as a result of enhanced licensing of their NK cells for killing by high-affinity KIR [75]. This argument cannot explain the experimental findings that the individuals who are homozygous for group I *HLA-C* and *KIR2DL3* can more efficiently clear needle-prick hepatitis C virus (HCV) infections as compared with the individuals who are homozygous for *KIR2DL1* and group II *HLA-C* [79]. As mentioned above, KIR2DL1/group II HLA-C interactions are stronger and mediate tighter inhibition of NK cells as compared with that mediated by the KIR2DL3/group I HLA-C. The implications of coinherited KIR and HLA genotypes are not restricted to resistance to viral infections (reviewed in ref. [51]). The individuals with relatively weakly inhibited NK cells would not only better control viral infections and other intracellular pathogens, but they would also be in a better position to control malignancy. On the other hand, they may be more susceptible to the development of various autoimmune diseases. Indeed, persons suffering from type I diabetes have been reported to carry a lower frequency of inhibitory *KIR/HLA* gene pairs, a higher frequency of activating *KIR* genes, and a higher frequency of KIR2DS2/ligand gene combinations [169, 170]. It is likely that a genetic imbalance between KIR and their ligands may have resulted in increased NK and CTL activities toward pancreatic antigens in these patients. According to this paradigm, the persons susceptible

to developing autoimmune diseases may control viral infections more efficiently. Consistent with this theme, it has been reported that HIV infection progresses more slowly toward AIDS in Zambian individuals suffering from the genetically determined, autoimmune spondylarthritis as compared with the spondylarthritis-free, HIV-infected persons [171]. The frequent presence of HLA-B27 in these patients may be a contributing factor in slowing the progression to AIDS in these patients.

## KIR-HLA INTERACTIONS IN RESISTANCE TO HIV INFECTION AND PROGRESSION TO AIDS

### Role of Bw4-binding KIR

Several genetic and functional studies have associated certain HLA allotypes with a rapid development of AIDS in HIV-infected persons. For example, the *HLA-B35 Px* allele has been associated with rapid progression toward AIDS, whereas *HLA-B57* and *HLA-B27* alleles have been associated with delayed progression. The HIV-infected persons carrying the protective alleles have slower decline of $CD4^+$ T cell counts, lower viral loads in their blood (viremia), and longer AIDS-free survival. The protective HLA alleles are also over-represented in HIV-infected persons, who can control HIV replication and do not develop AIDS for long periods of time (so-called natural controllers or long-term nonprogressors). The HLA-conferred protection from AIDS has been explained on the basis of the ability of these HLA allotypes to present immunodominant viral epitopes and induce broadly reactive polyclonal antiviral CTL responses [172–175]. It is noteworthy that these protective allotypes are seropositive for the Bw4 determinant. Furthermore, Bw4/Bw4 individuals have been shown to be relatively resistant to the development of AIDS as compared with Bw6/Bw6 individuals [75, 176]. As only Bw4-serospecific HLA allotypes have been shown to bind three-domain KIR, the question arises whether KIR/HLA-Bw4 interactions and NK cells could be involved in the Bw4-mediated resistance to the development of AIDS. Only a few studies have addressed this question and investigated the impact of KIR polymorphism as well as other *HLA/KIR* gene coinheritance on resistance to the development of AIDS. In this regard, Martin et al. [77] conducted investigations in a cohort of more than 1000 North American HIV-infected persons. They reported that an activating allele of the *KIR3DL1* gene, *KIR3DS1*, was associated with delayed progression to AIDS in humans coinheriting the *HLA-Bw4-I* alleles. In the absence of *KIR3DS1*, *HLA-Bw4-I* alleles were not associated with the protection. More surprisingly, *KIR3DS1* alone without its HLA ligand was associated with a relatively rapid progression of the disease. In a subsequent study, the group showed that coinherited *KIR3DS1* and *HLA-Bw4-I* alleles protected HIV-infected persons from viral burden in early stages of the infection and from opportunistic infections in late stages. Their coinheritance, however, afforded no protection from HIV-related malignancies [177]. As mentioned above, KIR3DL1 receptors bind the HLA-Bw4-I allotypes with higher affinity [70, 71]. Therefore, it can be argued that a high-affinity interaction between KIR3DS1 and HLA-Bw4-I allotypes may have resulted in more effective immune responses (through enhanced activation of NK cells and/or of T cells, were KIR3DS1 expressed on CTL in these patients). The problem in this interpretation is that in in vitro studies, although KIR3DL1 allotypes have been shown to bind HLA-Bw4 antigens in a peptide-dependent manner, KIR3DS1 does not bind any Bw4 allotype, even when complexed with the peptides that allow binding of KIR3DL1 allotypes [76, 78]. Alternately, a protein of viral or cellular origin, expressed de novo on the surface of HIV-infected cells, may have acted as a ligand for KIR3DS1. It is also possible that another unknown genetic determinant, which may be in linkage disequilibrium with *KIR3DS1*, may have been responsible for this protection. Furthermore, reasons behind the reported association of *KIR3DS1* with rapid progression of AIDS in the absence of its HLA ligand remain enigmatic. Another group of researchers could not corroborate these results in an ethnically different cohort of HIV-infected persons [178, 179]. They found no protective effect of coinherited *KIR3DS1* and *HLA-Bw4-I* genes on AIDS progression. Instead, they found that inheritance of two linked KIR genes (*KIR2DL2* and *KIR2DS2*; see Fig. 6) posed a greater risk of developing AIDS if their ligands were also present, and the presence of the *KIR2DL3* gene had a moderating effect on this risk. They also found that coinheritence of the genes for Bw6 and group II HLA-C allotypes was associated with rapid progression toward AIDS. The authors concluded that HLA and KIR affect HIV progression independently as well as synergistically. In another study on 88 HIV-infected individuals, it was shown that KIR3DL1 had a protective effect on the development of AIDS when coinherited with HLA-B*57 (an allotype with Ile at p80 [180]). An important drawback of these studies is that they did not characterize the *KIR3DS1* allele in the patients and control subjects. It is noteworthy that a mutant, nonfunctional form of *KIR3DS1* (**049N*) also exists [181, 182]. However, this mutant allele is rare. Martin et al. [181] reported later that their disease association data were not affected when they adjusted them for the presence of this allele. It may be relevant to mention here that KIR3DS1-expressing NK cells as well as T cells expand in viremic, HIV-infected persons [182]. These data suggest strongly that KIR3DS1 expression is relevant to this infection. Further studies are needed to pinpoint its exact role.

### Role of KIR3DL1 alleles

It is noteworthy that *KIR3DL1* has 59 alleles, 12 of which encode the activating allotypes (KIR3DS1), and 47 encode inhibitory (KIR3DL1) allotypes. Depending on their level of expression on the cell surface, Martin et al. [75] have divided the inhibitory allotypes into high expressors (*KIR3DL1*001*, **002*, **008*, **015*, **009*), low expressors (*KIR3DL1*005*, **007*), and no expressor (*KIR3DL1*004*). The last one is retained within cells as a result of its truncated, cytoplasmic tail. They presumed that the affinities of these allotypes for their MHC ligands correlated with their expression levels. They further demonstrated that highly expressing *KIR3DL1* alleles were protective when coinherited with *HLA-Bw4-I* alleles as compared with the low-expressing allotypes [75]. This study also showed relative protection afforded by low-expressing *KIR3DL1* alleles when coinherited with *HLA-Bw4-T* as

compared with the individuals homozygous for *Bw6* alleles. More surprisingly, *KIR3DL1*004* also provided protection, despite the fact that this allotype is not expressed on the cell surface. This shows that even an intracellularly retained KIR3DL1 allotype may have functional significance. These results from Martin et al. [75] suggest that the presence of a pair of inhibitory KIR3DL1 and its HLA ligand in HIV-infected persons affords protection from AIDS progression. Furthermore, the strength of the inhibition between the receptor-ligand pair correlates positively with the degree of protection. These results are not congruent with the paradigm that the KIR/HLA combinations that favored NK cell activation provided protection from viral infections and from the development of AIDS in HIV-infected individuals. The authors have argued that if NK cells were more strongly inhibited by KIR3DL1 receptor/ligand pairs, they would be more cytotoxic once they were relieved of their inhibition. The argument derives from the hypothesis that NK cells continue to express inhibitory KIR one after the other until they acquire sufficient inhibition to become self-tolerant. Thus, more tightly inhibited NK cells would be inherently more cytotoxic once their KIR/HLA-mediated inhibition is lost. The inhibition may be lost if the expression of HLA-Bw4 decreases on the surface of the virus-infected cells. Alternately, HLA-Bw4 may present a virus-derived peptide that may make it unrecognizable by the KIR3DL1 receptor. However, this argument cannot explain why coinheritence of *KIR/HLA* gene pairs, which inhibit NK cells rather loosely, or the inheritance of KIR without the genes for their HLA ligands protects humans from HIV and HCV infections [79, 183]. The authors have put forward another argument to explain their findings: In the presence of tightly inhibited NK cells, only a virus-specific, immune response is generated, avoiding a nonspecific, overall activation of the immune system. This response is more effective in suppressing HIV replication. Weakly inhibited NK cells may lead to a generalized activation of the immune response, which may cause immune-mediated pathology. However, there is no experimental evidence to support this argument. On the contrary, enhanced NK cell activation has been shown to induce better and stronger antigen-specific immune responses [184]. Furthermore, it does not explain results from several other studies in which relatively weakly inhibited NK cells (as a result of weak-affinity KIR/HLA interactions) have been shown to provide protection from viral infections as well as from tumors [51, 79, 178]. Even in the case of HIV infection, it has been reported that persons having weakly inhibited NK cells have reduced risk of contracting the infection. The study, conducted in African female sex workers, has shown that the inheritance of inhibitory *KIR* genes was protective from contracting HIV infection when the genes for their cognate MHC ligands were not coinherited. More specifically, *KIR2DL2/3* heterozygotes without group I HLA-C and KIR3DL1 homozygotes without HLA-Bw4 were relatively protected [183]. Furthermore, it has also been reported that the persons with KIR genotypes having more activating KIR genes were also relatively protected [183, 185]. In the case of i.v. drug users, stronger NK cell activities as well as a predominantly activating KIR repertoire (high KIR3DS1/KIR3DL1, NKG2C/NKG2A ratios, low expression of KIR3DL1, coinheritance of weakly inhibiting KIR/MHC pairs, i.e., KIR2DL3/HLA-C of group I) also protect from contracting HIV infection [185, 186]. Taken together, these studies suggest that weakly inhibited NK cells not only may slow progression of HIV infection toward AIDS but also may protect from contracting HIV infection. It is noteworthy that a hierarchy of KIR3DL1 allotypes for HLA-Bw4 binding has been described [149] that differs significantly from the one used by Martin et al. [75].

Concerning the impact of KIR3DS1 and its HLA ligands, Barbour et al. [187] have shown that the two genes affect AIDS progression independently from each other. The researchers analyzed viral load, $CD4^+$ T cell counts, and *KIR3DS1* and *HLA-Bw4* genotypes of a cohort of 255 treatment-naïve, HIV-infected persons during the first 2 years of infection. They found that the *KIR3DS1* and *HLA-Bw4-I* genes had distinct but independent effects on $CD4^+$ T cell counts and viral loads, respectively. They noted that *KIR3DS1*-positive, HIV-infected persons maintained $CD4^+$ T cells counts at higher levels as compared with the *KIR3DS1*-negative persons, irrespective of coinheritence of any *Bw4-I* alleles. The persons possessing *HLA-Bw4-I* alleles maintained lower viral loads all along the 2 years of the study period, irrespective of their *KIR3DS1* status. In the persons having the receptor and the ligand genes, the effects on $CD4^+$ T cell counts and viral load were simply additive and not synergistic. This study suggests a direct relationship between KIR3DS1 expression and $CD4^+$ T cell counts in HIV-infected persons. It may be interesting to investigate potential interactions between KIR3DS1-positive NK cells and $CD4^+$ T cells in humans. More recently, a direct role for an activating KIR, KIR3DS1, has been demonstrated in controlling HIV replication. In in vitro studies, KIR3DS1-positive NK cells inhibited HIV replication in HLA-Bw4-I-positive cell cultures in a contact and dose-dependent manner. The inhibition was significantly more as compared with KIR3DS1-negative NK cells [188]. Collectively, these studies do suggest a role of activated NK cells in controlling HIV infection. The caveat is that uncontrolled activation may contribute to immunopathogenesis.

It is noteworthy that HIV infections in humans have arisen relatively recently. The pathogen and its host have not had sufficient time to coevolve and eliminate deleterious genes from each other. Nevertheless, KIR and MHC antigens have evolved in humans under pressure from infectious agents, malignancy, and autoimmunity over millenia. Therefore, the impact of KIR genes, especially in combination with coinherited HLA genes on the susceptibility to HIV infection and development of AIDS in human populations, should be forthcoming. The studies conducted so far have yielded discordant results. The reasons for these discordant results may include variations in the pathogenicity of HIV viruses, treatment regimens, sample sizes, variable frequency of different genes in human populations, improper statistical models, etc. A part of the problem in formulating a uniform hypothesis regarding the impact of HLA/KIR interactions on the AIDS pathogenesis is the heterogeneous nature of the KIR with respect to their dependence on MHC-bound peptides. The two-domain KIR bind HLA-C and are affected by certain amino-acid side-chains at p7 and p8 of the MHC-bound nonamer peptides [83]. The three-domain KIR bind HLA-A and -B and are relatively

more discriminating between the peptides bound to their MHC ligand Bw4. Their recognition may have implications for antiviral CTL responses. For example, if an individual has HLA-Bw4 alleles, he/she will be expressing KIR3DL1 on a subset of his/her NK cells, which will recognize HLA-Bw4 complexed with endogenous self-peptides and will be tolerant to them. If the individual becomes infected, his/her cells may bind a foreign (antigenic) peptide to its HLA. If the new peptide-bound HLA is recognized by KIR3DL1, the cell still will be protected from NK cells but may be killed by antiviral CTL. If the new HLA-peptide complex is not recognized by KIR3DL1, the cell will no longer be protected from KIR3DL1-positive NK cells. The HLA-peptide complex may, however, be recognized by the CTL, and the infected cell will also be killed by the CTL. Thus, NK cells and CTL will eliminate the infected cell. Thus, the person coinheriting *KIR3DL1* and *HLA-Bw4* may be better equipped to eliminate HIV-infected cells compared with the person who is homozygous for *HLA-Bw6*. It is noteworthy that KIR and TCR bind their cognate MHC ligands with much different kinetics and thermodynamic properties [189, 190]. Therefore, if CTL and the KIR3DL1-positive NK cell recognize and bind the same Bw4-peptide complex, CTL may preclude the NK cell binding to the complex.

No KIR has been described that could bind HLA-Bw6 allotypes. Individuals bearing this HLA may be killed only by antiviral CTL (in the context of HLA-Bw6). It may explain why HLA-Bw4 and KIR3DL1 have synergistic effects in slowing down the progression of HIV toward AIDS (**Fig. 7**). As *HLA-Bw4* homozygous persons are not likely to lack *KIR3DL1*, this may also explain why *HLA-Bw4* homozygous individuals are relatively resistant to the development of AIDS as compared with *HLA-Bw6* homozygous ones [75, 176]. Furthermore, more protection may be afforded by high-affinity KIR3DL1 allotypes, as they may be more sensitive to the peptide requirements and therefore, may be less likely to recognize the HLA ligand if bound to a foreign peptide. However, at this point, it remains a speculation and needs to be proved by experimental data.

KIR3DS1 may be protective, as in normal uninfected persons, this receptor does not recognize autologous cells; otherwise, host NK cells may kill these autologous cells and would cause autoimmunity. In HIV-infected persons, a viral peptide may bind HLA-Bw4 and make it recognizable by the KIR3DS1. Under these conditions, KIR3DS1-positive NK cells will cause killing of the infected cell. Although KIR3DS1 and KIR3DL1 allotypes have more than 95% sequence identity at the amino-acid level in their ligand-binding, extracellular regions, they may still differ from each other in their three-dimentional structures. That is why a mAb DX9 binds KIR3DL1 but not KIR3DS1 [191]. Thus, it may not be surprising if KIR3DS1 and KIR3DL1 bind HLA-Bw4/peptide complexes.

One should not overlook the fact that KIR are also expressed on T cells, especially on the memory/effector phenotype. This is an area that needs to be addressed in HIV immunology. Furthermore, one should also keep in mind that Nef may be attenuating the protective effects of KIR3DL1/Bw4 interactions by down-regulating the expression of these MHC antigens.

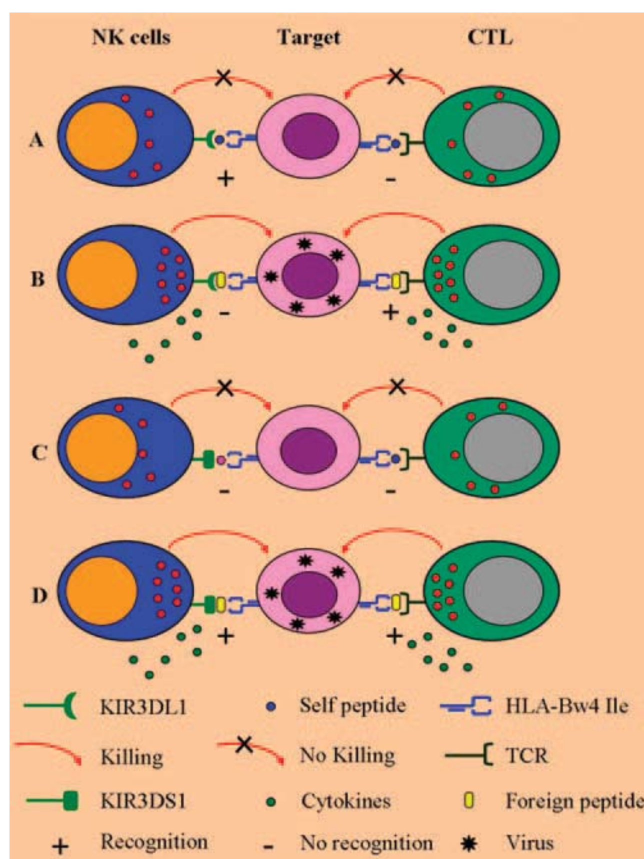


**Fig. 7.** NK cells with three-domain KIR behave like CTL in killing HIV-infected cells. (A) KIR3DL1 on NK cells recognize HLA-Bw4 with a self-peptide and are inhibited from killing normal, autologous cells. CTL do not recognize the self-peptide bound to the HLA and also do not kill the cell. (B) The virus-infected cell presents a virus-derived foreign peptide bound to HLA-Bw4. KIR3DL1 on the NK cell may not recognize the HLA with foreign peptide and is not inhibited from killing. The CTL may recognize the viral peptide bound to HLA-Bw4 and kill the infected cells. So, HIV-infected cells are more likely to be killed (from CTL as well as from NK cells). (C) KIR3DS1 expressed on NK cells does not bind to HLA-Bw4-I complexed with a self-peptide. CTL also do not recognize self-endogenous peptides and therefore, would not kill the healthy autologous cells. (D) A virus-infected cell presents a virus-derived foreign peptide bound to HLA-Bw4. It may be recognized by KIR3DS1 and be killed by KIR3DS1-positive NK cells. The CTL may also recognize the foreign peptide bound to HLA-Bw4 and therefore, will also kill the infected cell. Thus virus-infected, HLA-Bw4-positive cells are more likely to be killed (by virus-specific CTL and NK cells).

## Role of HLA-G-binding KIR

As stated above, nonclassical MHC class I antigens, HLA-G and -E, also act as ligands for some NKRs. HLA-G binds KIR2DL4, ILT-2, and possibly KIR2DL5. The gene for HLA-G shows limited polymorphism. It was demonstrated that an *HLA-G* allele, *HLA-G*0105N*, which codes for a nonfunctional mutant HLA-G, provides protection from HIV infection in East African female sex workers. On the other hand, inheritance of functionally active HLA-G alleles increased the risk of the infection in these workers [192]. It is noteworthy that HLA-G is expressed in the mucosal tissues of the female genital tract and may play a role in regulating local antiviral immunity. The authors interpreted the data by concluding that the mutant

antigen protected women, as it could not inhibit their NK cells in the reproductive tract of the HIV-exposed women. Of the NKRs, which could bind HLA-G, KIR2DL4 is expressed on all $CD56^+$ NK cells present in tissues in humans. However, as mentioned above in the KIR section, this receptor is not an authentic inhibitory KIR: It induces secretion of IFN-$\gamma$ but does not trigger cytotoxicity upon binding with its ligand [60, 63, 64]. Two other receptors, ILT-2 and possibly KIR2DL5, may also bind and inhibit NK cell functions. However, they are only expressed on a minor subset of NK cells. Notably, monocytes, macrophages, and DC abundantly express different ILT, including ILT-2, which preferentially bind and are inhibited by HLA-G [97]. Thus, in the persons having mutant HLA-G, activated monocytes, macrophages, and DC may play a greater role than NK cells. It has also been reported that the African women having a mutant *HLA-E* allele (*HLA-E*0103-G* or *HLA-E$^G$*) have four times less risk of contracting HIV infection as compared with the women with wild-type *HLA-E* [193]. It is noteworthy that the mutant *HLA-E* allele is expressed at lower levels and has decreased affinity for CD94/NKG2A receptors. It has also been shown that the sex worker women with HLA-E and HLA-G mutant genotypes (homozygous for *HLA-E$^G$* as well as heterozygous for HLA-*G*0105N*) had more than 12-fold decreased risk of contracting HIV infection [193]. These results implied a synergistic interaction between mutant HLA-E and HLA-G alleles in affording protection from HIV infection. These studies also suggest that blocking HLA-G and HLA-E interactions with NKR may enhance innate resistance to HIV.

Taken together, it can be postulated that coinheritance of genes for any NKR/ligand pair that weakens NK cell inhibition in the body and decreases activation threshold of NK and T cells is likely to provide protection from HIV and other viral infections. In this respect, the three-domain KIR behave as TCRs in recognizing MHC-bound peptides. If they fail to recognize the MHC-bound foreign peptide, they release the NK cell from inhibition. The NK cell will kill the virus-infected cell and so will do the virus-specific CTL. These two effector cells will be more effective in controlling HIV infection and delaying onset of AIDS in the persons who coinherit *KIR3DL1/HLA-Bw4* genes. If the three-domain KIR also recognize the foreign peptide, then the NK cell will be inhibited from killing the target cell. These results have implication for HIV vaccine strategies. Viral peptides, which are recognized by CTL, but not by KIR3DL1, may serve as better immunogens. They may arouse NK and CTL responses for killing the infected cells.

## PERSPECTIVE AND FUTURE DIRECTIONS

Advances made in understanding NK cell immunobiology have enabled us to appreciate the role of NK cells in host resistance to these infections. The functional activities of these cells are controlled by a multitude of inhibiting and activating receptors and coreceptors. Of these receptors, the genes encoding KIR and their HLA ligands are highly polymorphic. Therefore, it is not surprising that coinheritance of these genes and their epistatic interactions is important in determining the status of NK cell activation, overall immune reactivity, and innate genetic resistance of humans to viral infections, malignancy, and autoimmune diseases. Researchers have just begun unraveling the role of these interactions in these diseases.

In the case of HIV infection and AIDS, few studies conducted so far highlight the significance of *HLA* and *KIR* genes as well as of their interactions in determining our innate susceptibility to the infection and its progression to AIDS. Many of these studies lacked adequate sample sizes and did not take into account allelic variations. For example, all major KIR genes have allelic variants that encode nonfunctional receptors. Furthermore, different allotypes of a given KIR differ widely in their affinities for MHC ligands. Future studies should take into account the impact of these variants on the susceptibility/resistance of humans to the infection as well as on the rate of progression toward AIDS. The results could have a profound impact on our understanding of the role of NK cells in controlling HIV infection. In view of the large number of KIR genes, their alleles, differences in gene doses, and lack of knowledge about the ligands for activating KIR, the task of investigating the impact of KIR/HLA interactions on HIV infection is challenging but worth undertaking. The results would have enormous implications for the immunotherapy, prognosis, and vaccination of HIV infections. Such studies would require larger sample sizes, accurate clinical data, and proper stratification of study participants with respect to their ethnic backgrounds. Furthermore, the researchers must use models that include all KIR alleles and their ligand genes as well as all other known genetic determinants that affect host resistance to HIV infection.

In determining the role of genes for NKRs and those of their ligands, researchers have ignored the potential involvement of the ligands for KLR-D (NKG2D) receptors, i.e., MICA and MICB proteins, which exist in more than 50 and 20 allotypes, respectively. These allotypes vary in their affinity for NKG2D. The nature of the allotype(s) carried by an individual could greatly affect his/her NK cell ability to kill target cells. They have been shown to play a role in autoimmune diseases such as celiac disease, diabetes, etc.

Finally, research in this field is seriously hampered by lack of appropriate mAb to identify individual NKR genes and their allelic variants. Development of such reagents should be a priority. This would allow determining the level of expression of the genes at the protein level and supplement genetic data with more relevant protein data. Furthermore, these antibodies could serve as important tools for manipulating receptor/ligand interactions for therapeutic purposes.

## NOTE ADDED IN PROOF

A recent publication showed significantly enhanced frequency of *KIR3DS1* homozygotes but not of the combination of the *KIR3D* gene with *HLA-Bw4* or *HLA-Bw4-I* genes in HIV-exposed uninfected individuals compared to HIV-infected ones [194].

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## REFERENCES

1. Iannello, A., Debbeche, O., Martin, E., Attalah, L. H., Samarani, S., Ahmad, A. (2006) Viral strategies for evading antiviral cellular immune responses of the host. *J. Leukoc. Biol.* **79,** 16–35.
2. Andoniou, C. E., Andrews, D. M., Degli-Esposti, M. A. (2006) Natural killer cells in viral infection: more than just killers. *Immunol. Rev.* **214,** 239–250.
3. Orange, J. S. (2002) Human natural killer cell deficiencies and susceptibility to infection. *Microbes Infect.* **4,** 1545–1558.
4. Di Santo, J. P., Vosshenrich, C. A. J. (2006) Bone marrow versus thymic pathways of natural killer cell development. *Immunol. Rev.* **214,** 35–46.
5. Freud, A. G., Caligiuri, M. A. (2006) Human natural killer cell development. *Immunol. Rev.* **214,** 56–72.
6. Bendelac, A., Savage, P. B., Teyton, L. (2007) The biology of NKT cells. *Annu. Rev. Immunol.* **25,** 297–336.
7. Van Kaer, L. (2007) NKT cells: T lymphocytes with innate effector functions. *Curr. Opin. Immunol.* **19,** 354–364.
8. Cooper, M. A., Fehniger, T. A., Turner, S. C., Chen, K. S., Ghaheri, B. A., Ghayur, T., Carson, W. E., Caligiuri, M. A. (2001) Human natural killer cells: a unique innate immunoregulatory role for the CD56bright subset. *Blood* **97,** 3146–3151.
9. Farag, S. S., Fehniger, T. A., Ruggeri, L., Velardi, A., Caligiuri, M. A. (2002) Natural killer cell receptors: new biology and insights into the graft-versus-leukemia effect. *Blood* **100,** 1935–1947.
10. Hanna, J., Mussaffi, H., Steuer, G., Hanna, S., Deeb, M., Blau, H., Arnon, T. I., Weizman, N., Mandelboim, O. (2005) Functional aberrant expression of CCR2 receptor on chronically activated NK cells in patients with TAP-2 deficiency. *Blood* **106,** 3465–3473.
11. Keskin, D. B., Allan, D. S. J., Rybalov, B., Andzelm, M. M., Stern, J. N. H., Kopcow, H. D., Koopman, L. A., Strominger, J. L. (2007) TGFβ promotes conversion of CD16+ peripheral blood NK cells into CD16− NK cells with similarities to decidual NK cells. *Proc. Natl. Acad. Sci. USA* **104,** 3378–3383.
12. Romagnani, C., Juelke, K., Falco, M., Morandi, B., D'Agostino, A., Costa, R., Ratto, G., Forte, G., Carrega, P., Lui, G., Conte, R., Strowig, T., Moretta, A., Munz, C., Thiel, A., Moretta, L., Ferlazzo, G. (2007) CD56brightCD16− killer Ig-like receptor− NK cells display longer telomeres and acquire features of CD56dim NK cells upon activation. *J. Immunol.* **178,** 4947–4955.
13. Takahashi, E., Kuranaga, N., Satoh, K., Habu, Y., Shinomiya, N., Asano, T., Seki, S., Hayakawa, M. (2007) Induction of CD16+ CD56bright NK cells with antitumour cytotoxicity not only from CD16− CD56bright NK cells but also from CD16− CD56dim NK cells. *Scand. J. Immunol.* **65,** 126–138.
14. Ahmad, A., Ahmad, R. (2003) HIV evasion of host NK cell response and novel ways of its countering and boosting anti-HIV immunity. *Curr. HIV Res.* **1,** 295–307.
15. Guidotti, L. G., Chisari, F. V. (2001) Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu. Rev. Immunol.* **19,** 65–91.
16. Zhang, A. L., Colmenero, P., Purath, U., Teixeira de Matos, C., Hueber, W., Klareskog, L., Tarner, I. H., Engleman, E. G., Soderstrom, K. (2007) Natural killer cells trigger differentiation of monocytes into dendritic cells. *Blood* **110,** 2484–2493.
17. Geldhof, A. B., Van Ginderachter, J. A., Liu, Y., Noel, W., Raes, G., De Baetselier, P. (2002) Antagonistic effect of NK cells on alternatively activated monocytes: a contribution of NK cells to CTL generation. *Blood* **100,** 4049–4058.
18. Nedvetzki, S., Sowinski, S., Eagle, R. A., Harris, J., Vely, F., Pende, D., Trowsdale, J., Vivier, E., Gordon, S., Davis, D. M. (2007) Reciprocal regulation of human natural killer cells and macrophages associated with distinct immune synapses. *Blood* **109,** 3776–3785.
19. Chan, C. W., Crafton, E., Fan, H-N., Flook, J., Yoshimura, K., Skarica, M., Brockstedt, D., Dubensky, T. W., Stins, M. F., Lanier, L. L., Pardoll, D. M., Housseau, F. (2006) Interferon-producing killer dendritic cells provide a link between innate and adaptive immunity. *Nat. Med.* **12,** 207–213.
20. Hanna, J., Mandelboim, O. (2007) When killers become helpers. *Trends Immunol.* **28,** 201–206.
21. Taieb, J., Chaput, N., Menard, C., Apetoh, L., Ullrich, E., Bonmort, M., Pequignot, M., Casares, N., Terme, M., Flament, C., Opolon, P., Lecluse, Y., Metivier, D., Tomasello, E., Vivier, E., Ghiringhelli, F., Martin, F., Klatzmann, D., Poynard, T., Tursz, T., Raposo, G., Yagita, H., Ryffel, B., Kroemer, G., Zitvogel, L. (2006) A novel dendritic cell subset involved in tumor immunosurveillance. *Nat. Med.* **12,** 214–219.
22. Vosshenrich, C. A. J., Lesjean-Pottier, S., Hasan, M., Richard-Le Goff, O., Corcuff, E., Mandelboim, O., Di Santo, J. P. (2007) CD11cloB220+ interferon-producing killer dendritic cells are activated natural killer cells. *J. Exp. Med.* **204,** 2569–2578.
23. Mailliard, R. B., Alber, S. M., Shen, H., Watkins, S. C., Kirkwood, J. M., Herberman, R. B., Kalinski, P. (2005) IL-18-induced CD83+CCR7+ NK helper cells. *J. Exp. Med.* **202,** 941–953.
24. Tanaka, F., Hashimoto, W., Okamura, H., Robbins, P. D., Lotze, M. T., Tahara, H. (2000) Rapid generation of potent and tumor-specific cytotoxic T lymphocytes by interleukin 18 using dendritic cells and natural killer cells. *Cancer Res.* **60,** 4838–4844.
25. Lu, L., Ikizawa, K., Hu, D., Werneck, M. B. F., Wucherpfennig, K. W., Cantor, H. (2007) Regulation of activated CD4+ T cells by NK cells via the Qa-1-NKG2A inhibitory pathway. *Immunity* **26,** 593–604.
26. Vankayalapati, R., Klucar, P., Wizel, B., Weis, S. E., Samten, B., Safi, H., Shams, H., Barnes, P. F. (2004) NK cells regulate CD8+ T cell effector function in response to an intracellular pathogen. *J. Immunol.* **172,** 130–137.
27. Gao, N., Dang, T., Yuan, D. (2001) IFN-γ-dependent and -independent initiation of switch recombination by NK cells. *J. Immunol.* **167,** 2011–2018.
28. Takahashi, K., Miyake, S., Kondo, T., Terao, K., Hatakenaka, M., Hashimoto, S., Yamamura, T. (2001) Natural killer type 2 bias in remission of multiple sclerosis. *J. Clin. Invest.* **107,** R23–R29.
29. Wei, H., Zhang, J., Xiao, W., Feng, J., Sun, R., Tian, Z. (2005) Involvement of human natural killer cells in asthma pathogenesis: natural killer 2 cells in type 2 cytokine predominance. *J. Allergy Clin. Immunol.* **115,** 841–847.
30. O'Leary, J. G., Goodarzi, M., Drayton, D. L., von Andrian, U. H. (2006) T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nat. Immunol.* **7,** 507–516.
31. Hiby, S. E., Walker, J. J., O'Shaughnessy, K. M., Redman, C. W. G., Carrington, M., Trowsdale, J., Moffett, A. (2004) Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *J. Exp. Med.* **200,** 957–965.
32. Yan, W. H., Lin, A., Chen, B. G., Zhou, M. Y., Dai, M. Z., Chen, X J., Gan, L. H., Zhu, M., Shi, W. W., Li, B. L. (2007) Possible roles of KIR2DL4 expression on uNK cells in human pregnancy. *Am. J. Reprod. Immunol.* **57,** 233–242.
33. Biron, C. A., Nguyen, K. B., Pien, G. C., Cousens, L. P., Salazar-Mather, T. P. (1999) Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu. Rev. Immunol.* **17,** 189–220.
34. Ahmad, A., Ahmad, R., Iannello, A., Toma, E., Morisset, R., Sindhu, S. (2005) IL-15 and HIV infection: lessons for immunotherapy and vaccination. *Curr. HIV Res.* **3,** 261–270.
35. Chalifour, A., Jeannin, P., Gauchat, J-F., Blaecke, A., Malissard, M., N'Guyen, T., Thieblemont, N., Delneste, Y. (2004) Direct bacterial protein PAMP recognition by human NK cells involves TLRs and triggers {α}-defensin production. *Blood* **104,** 1778–1783.
36. Schmidt, K. N., Leung, B., Kwong, M., Zarember, K. A., Satyal, S., Navas, T. A., Wang, F., Godowski, P. J. (2004) APC-independent activation of NK cells by the Toll-like receptor 3 agonist double-stranded RNA. *J. Immunol.* **172,** 138–143.
37. Alter, G., Suscovich, T. J., Teigen, N., Meier, A., Streeck, H., Brander, C., Altfeld, M. (2007) Single-stranded RNA derived from HIV-1 serves as a potent activator of NK cells. *J. Immunol.* **178,** 7658–7666.
38. Baratin, M., Roetynck, S., Lepolard, C., Falk, C., Sawadogo, S., Uematsu, S., Akira, S., Ryffel, B., Tiraby, J-G., Alexopoulou, L., Kirschning, C. J., Gysin, J., Vivier, E., Ugolini, S. (2005) Natural killer cell and macrophage cooperation in MyD88-dependent innate responses to *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. USA* **102,** 14747–14752.
39. Hart, O. M., Athie-Morales, V., O'Connor, G. M., Gardiner, C. M. (2005) TLR7/8-mediated activation of human NK cells results in accessory cell-dependent IFN-{γ} production. *J. Immunol.* **175,** 1636–1642.

40. Mandelboim, O., Lieberman, N., Lev, M., Paul, L., Arnon, T. I., Bushkin, Y., Davis, D. M., Strominger, J. L., Yewdell, J. W., Porgador, A. (2001) Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells. *Nature* **409,** 1055–1060.
41. Arase, H., Arase, N., Saito, T. (1995) Fas-mediated cytotoxicity by freshly isolated natural killer cells. *J. Exp. Med.* **181,** 1235–1238.
42. Zamai, L., Ahmad, M., Bennett, I. M., Azzoni, L., Alnemri, E. S., Perussia, B. (1998) Natural killer (NK) cell-mediated cytotoxicity: differential use of TRAIL and Fas ligand by immature and mature primary human NK cells. *J. Exp. Med.* **188,** 2375–2380.
43. Ahmad, A., Alvarez, F. (2004) Role of NK and NKT cells in the immunopathogenesis of HCV-induced hepatitis. *J. Leukoc. Biol.* **76,** 743–759.
44. Bryceson, Y. T., March, M. E., Barber, D. F., Ljunggren, H-G., Long, E. O. (2005) Cytolytic granule polarization and degranulation controlled by different receptors in resting NK cells. *J. Exp. Med.* **202,** 1001–1012.
45. MacFarlane IV, A. W., Campbell, K. (2006) Signal transduction in natural killer cells. *Curr. Top. Microbiol. Immunol.* **298,** 23–57.
46. Davis, D. M., Dustin, M. L. (2004) What is the importance of the immunological synapse? *Trends Immunol.* **25,** 323–327.
47. Wulfing, C., Purtic, B., Klem, J., Schatzle, J. D. (2003) Stepwise cytoskeletal polarization as a series of checkpoints in innate but not adaptive cytolytic killing. *Proc. Natl. Acad. Sci. USA* **100,** 7767–7772.
48. Davis, D. M. (2007) Intercellular transfer of cell-surface proteins is common and can affect many stages of an immune response. *Nat. Rev. Immunol.* **7,** 238–243.
49. McCann, F. E., Eissmann, P., Onfelt, B., Leung, R., Davis, D. M. (2007) The activating NKG2D ligand MHC class I-related chain a transfers from target cells to NK cells in a manner that allows functional consequences. *J. Immunol.* **178,** 3418–3426.
50. Onfelt, B., Purbhoo, M. A., Nedvetzki, S., Sowinski, S., Davis, D. M. (2005) Long-distance calls between cells connected by tunneling nanotubules. *Sci. STKE* **2005,** pe55.
51. Khakoo, S. I., Carrington, M. (2006) KIR and disease: a model system or system of models? *Immunol. Rev.* **214,** 186–201.
52. Long, E. O. (1999) Regulation of immune responses through inhibitory receptors. *Annu. Rev. Immunol.* **17,** 875–904.
53. Lanier, L. L. (2005) NK cell recognition. *Annu. Rev. Immunol.* **23,** 225–274.
54. Lanier, L. L. (1998) NK cell receptors. *Annu. Rev. Immunol.* **16,** 359–393.
55. Kim, J., Chwae, Y. J., Kim, M. Y., Choi, I. H., Park, J. H., Kim, S. J. (1997) Molecular basis of HLA-C recognition by p58 natural killer cell inhibitory receptors. *J. Immunol.* **159,** 3875–3882.
56. Winter, C. C., Long, E. (1997) A single amino acid in the p58 killer cell inhibitory receptor controls the ability of natural killer cells to discriminate between the two groups of HLA-C allotypes. *J. Immunol.* **158,** 4026–4028.
57. Winter, C. C., Gumperz, J. E., Parham, P., Long, E. O., Wagtmann, N. (1998) Direct binding and functional transfer of NK cell inhibitory receptors reveal novel patterns of HLA-C allotype recognition. *J. Immunol.* **161,** 571–577.
58. Biassoni, R., Falco, M., Cambiaggi, A., Costa, P., Verdiani, S., Pende, D., Conte, R., Di Donato, C., Parham, P., Moretta, L. (1995) Amino acid substitutions can influence the natural killer (NK)-mediated recognition of HLA-C molecules. Role of serine-77 and lysine-80 in the target cell protection from lysis mediated by "group 2" or "group 1" NK clones. *J. Exp. Med.* **182,** 605–609.
59. Faure, M., Long, E. O. (2002) KIR2DL4 (CD158d), an NK cell-activating receptor with inhibitory potential. *J. Immunol.* **168,** 6208–6214.
60. Goodridge, J. P., Witt, C. S., Christiansen, F. T., Warren, H. S. (2003) KIR2DL4 (CD158d) genotype influences expression and function in NK cells. *J. Immunol.* **171,** 1768–1774.
61. Kikuchi-Maki, A., Catina, T. L., Campbell, K. S. (2005) Cutting edge: KIR2DL4 transduces signals into human NK cells through association with the Fc receptor {γ} protein. *J. Immunol.* **174,** 3859–3863.
62. Kikuchi-Maki, A., Yusa, S-i., Catina, T. L., Campbell, K. S. (2003) KIR2DL4 is an IL-2-regulated NK cell receptor that exhibits limited expression in humans but triggers strong IFN-{γ} production. *J. Immunol.* **171,** 3415–3425.
63. Rajagopalan, S., Bryceson, Y. T., Kuppusamy, S. P., Geraghty, D. E., van der Meer, A., Joosten, I., Long, E. O. (2006) Activation of NK cells by an endocytosed receptor for soluble HLA-G. *PLoS Biol.* **4,** e9.
64. Rajagopalan, S., Fu, J., Long, E. O. (2001) Cutting edge: induction of IFN-{γ} production but not cytotoxicity by the killer cell Ig-like receptor KIR2DL4 (CD158d) in resting NK cells. *J. Immunol.* **167,** 1877–1881.
65. Sargent, I. L., Borzychowski, A. M., Redman, C. W. G. (2006) NK cells and human pregnancy—an inflammatory view. *Trends Immunol.* **27,** 399–404.
66. Gomez-Lozano, N., Pablo, R., Puente, S., Vilches, C. (2003) Recognition of HLA-G by the NK cell receptor KIR2DL4 is not essential for human reproduction. *Eur. J. Immunol.* **33,** 639–644.
67. Gómez-Lozano, N., Gardiner, C., Parham, P., Vilches, C. (2002) Some human KIR haplotypes contain two KIR2DL5 genes: KIR2DL5A and KIR2DL5B. *Immunogenetics* **54,** 314–319.
68. Estefania, E., Flores, R., Gomez-Lozano, N., Aguilar, H., Lopez-Botet, M., Vilches, C. (2007) Human KIR2DL5 is an inhibitory receptor expressed on the surface of NK and T lymphocyte subsets. *J. Immunol.* **178,** 4402–4410.
69. Yusa, S-i., Catina, T. L., Campbell, K. S. (2004) KIR2DL5 can inhibit human NK cell activation via recruitment of Src homology region 2-containing protein tyrosine phosphatase-2 (SHP-2). *J. Immunol.* **172,** 7385–7392.
70. Cella, M., Longo, A., Ferrara, G., Strominger, J., Colonna, M. (1994) NK3-specific natural killer cells are selectively inhibited by Bw4-positive HLA alleles with isoleucine 80. *J. Exp. Med.* **180,** 1235–1242.
71. Rojo, S., Wagtmann, N., Long, E. (1997) Binding of a soluble p70 killer cell inhibitory receptor to HLA-B*5101: requirement for all three p70 immunoglobulin domains. *Eur. J. Immunol.* **27,** 568–571.
72. Malnati, M. S., Peruzzi, M., Parker, K. C., Biddison, W. E., Ciccone, E., Moretta, A., Long, E. O. (1995) Peptide specificity in the recognition of MHC class I by natural killer cell clones. *Science* **267,** 1016–1018.
73. O'Connor, G. M., Guinan, K. J., Cunningham, R. T., Middleton, D., Parham, P., Gardiner, C. M. (2007) Functional polymorphism of the KIR3DL1/S1 receptor on human NK cells. *J. Immunol.* **178,** 235–241.
74. Thananchai, H., Gillespie, G., Martin, M. P., Bashirova, A., Yawata, N., Yawata, M., Easterbrook, P., McVicar, D. W., Maenaka, K., Parham, P., Carrington, M., Dong, T., Rowland-Jones, S. (2007) Cutting edge: allele-specific and peptide-dependent interactions between KIR3DL1 and HLA-A and HLA-B. *J. Immunol.* **178,** 33–37.
75. Martin, M. P., Qi, Y., Gao, X., Yamada, E., Martin, J. N., Pereyra, F., Colombo, S., Brown, E. E., Shupert, W. L., Phair, J., Goedert, J. J., Buchbinder, S., Kirk, G. D., Telenti, A., Connors, M., O'Brien, S. J., Walker, B. D., Parham, P., Deeks, S. G., McVicar, D. W., Carrington, M. (2007) Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat. Genet.* **39,** 733–740.
76. O'Connor, G. M., Guinan, K. J., Cunningham, R. T., Middleton, D., Parham, P., Gardiner, C. M. (2007) Functional polymorphism of the KIR3DL1/S1 receptor on human NK cells. *J. Immunol.* **178,** 235–241.
77. Martin, M. P., Gao, X., Lee, J-H., Nelson, G. W., Detels, R., Goedert, J. J., Buchbinder, S., Hoots, K., Vlahov, D., Trowsdale, J., Wilson, M., O'Brien, S. J., Carrington, M. (2002) Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. *Nat. Genet.* **31,** 429–434.
78. Carr, W. H., Rosen, D. B., Arase, H., Nixon, D. F., Michaelsson, J., Lanier, L. L. (2007) Cutting edge: KIR3DS1, a gene implicated in resistance to progression to AIDS, encodes a DAP12-associated receptor expressed on NK cells that triggers NK cell activation. *J. Immunol.* **178,** 647–651.
79. Khakoo, S. I., Thio, C. L., Martin, M. P., Brooks, C. R., Gao, X., Astemborski, J., Cheng, J., Goedert, J. J., Vlahov, D., Hilgartner, M., Cox, S., Little, A-M., Alexander, G. J., Cramp, M. E., O'Brien, S. J., Rosenberg, W. M. C., Thomas, D. L., Carrington, M. (2004) HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science* **305,** 872–874.
80. Katz, G., Gazit, R., Arnon, T. I., Gonen-Gross, T., Tarcic, G., Markel, G., Gruda, R., Achdout, H., Drize, O., Merims, S., Mandelboim, O. (2004) MHC class I-independent recognition of NK-activating receptor KIR2DS4. *J. Immunol.* **173,** 1819–1825.
81. Arase, H., Mocarski, E. S., Campbell, A. E., Hill, A. B., Lanier, L. L. (2002) Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. *Science* **296,** 1323–1326.
82. Desrosiers, M-P., Kielczewska, A., Loredo-Osti, J. C., Adam, S. G., Makrigiannis, A. P., Lemieux, S., Pham, T., Lodoen, M. B., Morgan, K., Lanier, L. L., Vidal, S. M. (2005) Epistasis between mouse Klra and major histocompatibility complex class I loci is associated with a new mechanism of natural killer cell-mediated innate resistance to cytomegalovirus infection. *Nat. Genet.* **37,** 593–599.
83. Rajagopalan, S., Long, E. O. (1997) The direct binding of a p58 killer cell inhibitory receptor to human histocompatibility leukocyte antigen (HLA)-Cw4 exhibits peptide selectivity. *J. Exp. Med.* **185,** 1523–1528.
84. Liberatore, C., Capanni, M., Albi, N., Volpi, I., Urbani, E., Ruggeri, L., Mencarelli, A., Grignani, F., Velardi, A. (1999) Natural killer cell-

mediated lysis of autologous cells modified by gene therapy. *J. Exp. Med.* **189,** 1855–1862.
85. Diefenbach, A., Raulet, D. H. (2001) Strategies for target cell recognition by natural killer cells. *Immunol. Rev.* **181,** 170–184.
86. Kim, D-K., Kabat, J., Borrego, F., Sanni, T. B., You, C-h., Coligan, J. E. (2004) Human NKG2F is expressed and can associate with DAP12. *Mol. Immunol.* **41,** 53–62.
87. Takaki, R., Watson, S. R., Lanier, L. L. (2006) DAP12: an adapter protein with dual functionality. *Immunol. Rev.* **214,** 118–129.
88. Borrego, F., Ulbrecht, M., Weiss, E. H., Coligan, J. E., Brooks, A. G. (1998) Recognition of human histocompatibility leukocyte antigen (HLA)-E complexed with HLA class I signal sequence-derived peptides by CD94/NKG2 confers protection from natural killer cell-mediated lysis. *J. Exp. Med.* **187,** 813–818.
89. Braud, V. M., Allan, D. S. J., O'Callaghan, C. A., Soderstrom, K., D'Andrea, A., Ogg, G. S., Lazetic, S., Young, N. T., Bell, J. I., Phillips, J. H., Lanier, L. L., McMichael, A. J. (1998) HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* **391,** 795–799.
90. Lee, N., Llano, M., Carretero, M., Ishitani, A., Navarro, F., Lopez-Botet, M., Geraghty, D. E. (1998) HLA-E is a major ligand for the natural killer inhibitory receptor CD94/NKG2A. *Proc. Natl. Acad. Sci. USA* **95,** 5199–5204.
91. Leibson, P. J. (1998) Cytotoxic lymphocyte recognition of HLA-E: utilizing a nonclassical window to peer into classical MHC. *Immunity* **9,** 289–294.
92. Vance, R. E., Jamieson, A. M., Raulet, D. H. (1999) Recognition of the class Ib molecule Qa-1b by putative activating receptors CD94/NKG2C and CD94/NKG2E on mouse natural killer cells. *J. Exp. Med.* **190,** 1801–1812.
93. Tomasec, P., Braud, V. M., Rickards, C., Powell, M. B., McSharry, B. P., Gadola, S., Cerundolo, V., Borysiewicz, L. K., McMichael, A. J., Wilkinson, G. W. (2000) Surface expression of HLA-E, an inhibitor of natural killer cells, enhanced by human cytomegalovirus gpUL40. *Science* **287,** 1031.
94. Coupel, S., Moreau, A., Hamidou, M., Horejsi, V., Soulillou, J-P., Charreau, B. (2007) Expression and release of soluble HLA-E is an immunoregulatory feature of endothelial cell activation. *Blood* **109,** 2806–2814.
95. Matsumoto, N., Ribaudo, R. K., Abastado, J-P., Margulies, D. H., Yokoyama, W. M. (1998) The lectin-like NK cell receptor Ly-49A recognizes a carbohydrate-independent epitope on its MHC class I ligand. *Immunity* **8,** 245–254.
96. Long, E. O. (2002) Versatile signaling through NKG2D. *Nat. Immunol.* **3,** 1119–1120.
97. Shiroishi, M., Tsumoto, K., Amano, K., Shirakihara, Y., Colonna, M., Braud, V. M., Allan, D. S. J., Makadzange, A., Rowland-Jones, S., Willcox, B., Jones, E. Y., van der Merwe, P. A., Kumagai, I., Maenaka, K. (2003) Human inhibitory receptors Ig-like transcript 2 (ILT2) and ILT4 compete with CD8 for MHC class I binding and bind preferentially to HLA-G. *Proc. Natl. Acad. Sci. USA* **100,** 8856–8861.
98. Young, N. T., Uhrberg, M., Phillips, J. H., Lanier, L. L., Parham, P. (2001) Differential expression of leukocyte receptor complex-encoded Ig-like receptors correlates with the transition from effector to memory CTL. *J. Immunol.* **166,** 3933–3941.
99. Giustiniani, J., Marie-Cardine, A., Bensussan, A. (2007) A soluble form of the MHC class I-specific CD160 receptor is released from human activated NK lymphocytes and inhibits cell-mediated cytotoxicity. *J. Immunol.* **178,** 1293–1300.
100. Wu, J., Song, Y., Bakker, A. B., Bauer, S., Spies, T., Lanier, L. L., Phillips, J. H. (1999) An activating immunoreceptor complex formed by NKG2D and DAP10. *Science* **285,** 730–732.
101. Markiewicz, M. A., Carayannopoulos, L. N., Naidenko, O. V., Matsui, K., Burack, W. R., Wise, E. L., Fremont, D. H., Allen, P. M., Yokoyama, W. M., Colonna, M., Shaw, A. S. (2005) Costimulation through NKG2D enhances murine CD8+ CTL function: similarities and differences between NKG2D and CD28 costimulation. *J. Immunol.* **175,** 2825–2833.
102. Bauer, S., Groh, V., Wu, J., Steinle, A., Phillips, J. H., Lanier, L. L., Spies, T. (1999) Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* **285,** 727–729.
103. Stephens, H. A. (2001) MICA and MICB genes: can the enigma of their polymorphism be resolved? *Trends Immunol.* **22,** 378–385.
104. Cerwenka, A., Bakker, A. B. H., McClanahan, T., Wagner, J., Wu, J., Phillips, J. H., Lanier, L. L. (2000) Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. *Immunity* **12,** 721–727.
105. Diefenbach, A., Jamieson, A. M., Liu, S. D., Shastri, N., Raulet, D. H. (2000) Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages. *Nat. Immunol.* **1,** 119–126.
106. Gasser, S., Orsulic, S., Brown, E. J., Raulet, D. H. (2005) The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. *Nature* **436,** 1186–1190.
107. Groh, V., Rhinehart, R., Randolph-Habecker, J., Topp, M. S., Riddell, S. R., Spies, T. (2001) Costimulation of CD8[α][β] T cells by NKG2D via engagement by MIC induced on virus-infected cells. *Nat. Immunol.* **2,** 255–260.
108. Salih, H. R., Antropius, H., Gieseke, F., Lutz, S. Z., Kanz, L., Rammensee, H-G., Steinle, A. (2003) Functional expression and release of ligands for the activating immunoreceptor NKG2D in leukemia. *Blood* **102,** 1389–1396.
109. Salih, H. R., Goehlsdorf, D., Steinle, A. (2006) Release of MICB molecules by tumor cells: mechanism and soluble MICB in sera of cancer patients. *Hum. Immunol.* **67,** 188–195.
110. Bashirova, A. A., Martin, M. P., McVicar, D. W., Carrington, M. (2006) The killer immunoglobulin-like receptor gene cluster: tuning the genome for defense. *Annu. Rev. Genomics Hum. Genet.* **7,** 277–300.
111. González, S., Groh, V., Spies, T. (2006) Immunobiology of human NKG2D and its ligands. *Curr. Top. Microbiol. Immunol.* **298,** 121–138.
112. Dhanji, S., Teh, S-J., Oble, D., Priatel, J. J., Teh, H-S. (2004) Self-reactive memory-phenotype CD8 T cells exhibit both MHC-restricted and non-MHC-restricted cytotoxicity: a role for the T-cell receptor and natural killer cell receptors. *Blood* **104,** 2116–2123.
113. Bottino, C., Castriconi, R., Pende, D., Rivera, P., Nanni, M., Carnemolla, B., Cantoni, C., Grassi, J., Marcenaro, S., Reymond, N., Vitale, M., Moretta, L., Lopez, M., Moretta, A. (2003) Identification of PVR (CD155) and nectin-2 (CD112) as cell surface ligands for the human DNAM-1 (CD226) activating molecule. *J. Exp. Med.* **198,** 557–567.
114. Hamerman, J. A., Ogasawara, K., Lanier, L. L. (2004) Cutting edge: Toll-like receptor signaling in macrophages induces ligands for the NKG2D receptor. *J. Immunol.* **172,** 2001–2005.
115. Saikali, P., Antel, J. P., Newcombe, J., Chen, Z., Freedman, M., Blain, M., Cayrol, R., Prat, A., Hall, J. A., Arbour, N. (2007) NKG2D-mediated cytotoxicity toward oligodendrocytes suggests a mechanism for tissue injury in multiple sclerosis. *J. Neurosci.* **27,** 1220–1228.
116. Biassoni, R., Cantoni, C., Pende, D., Sivori, S., Parolini, S., Vitale, M., Bottino, C., Moretta, A. (2001) Human natural killer cell receptors and co-receptors. *Immunol. Rev.* **181,** 203–214.
117. Moretta, A., Bottino, C., Vitale, M., Pende, D., Cantoni, C., Mingari, M. C., Biassoni, R., Moretta, L. (2001) Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. *Annu. Rev. Immunol.* **19,** 197–223.
118. Moretta, L., Bottino, C., Pende, D., Vitale, M., Mingari, M. C., Moretta, A. (2004) Different checkpoints in human NK-cell activation. *Trends Immunol.* **25,** 670–676.
119. Arnon, T. I., Achdout, H., Lieberman, N., Gazit, R., Gonen-Gross, T., Katz, G., Bar-Ilan, A., Bloushtain, N., Lev, M., Joseph, A., Kedar, E., Porgador, A., Mandelboim, O. (2004) The mechanisms controlling the recognition of tumor- and virus-infected cells by NKp46. *Blood* **103,** 664–672.
120. Gazit, R., Garty, B-Z., Monselise, Y., Hoffer, V., Finkelstein, Y., Markel, G., Katz, G., Hanna, J., Achdout, H., Gruda, R., Gonen-Gross, T., Mandelboim, O. (2004) Expression of KIR2DL1 on the entire NK cell population: a possible novel immunodeficiency syndrome. *Blood* **103,** 1965–1966.
121. Aldemir, H., Prod'homme, V., Dumaurier, M-J., Retiere, C., Poupon, G., Cazareth, J., Bihl, F., Braud, V. M. (2005) Cutting edge: lectin-like transcript 1 is a ligand for the CD161 receptor. *J. Immunol.* **175,** 7791–7795.
122. Mathew, P. A., Chuang, S., Vaidya, S., Kumaresan, P., Boles, K., Pham, H. (2004) The LLT1 receptor induces IFN-γ production by human natural killer cells. *Mol. Immunol.* **40,** 1157–1163.
123. Veillette, A. (2006) NK cell regulation by SLAM family receptors and SAP-related adapters. *Immunol. Rev.* **214,** 22–34.
124. Nichols, K. E., Hom, J., Gong, S-Y., Ganguly, A., Ma, C. S., Cannons, J. L., Tangye, S. G., Schwartzberg, P. L., Koretzky, G. A., Stein, P. L. (2005) Regulation of NKT cell development by SAP, the protein defective in XLP. *Nat. Med.* **11,** 340–345.
125. Benoit, L., Wang, X., Pabst, H. F., Dutz, J., Tan, R. (2000) Cutting edge: defective NK cell activation in X-linked lymphoproliferative disease. *J. Immunol.* **165,** 3549–3553.
126. Parolini, S., Bottino, C., Falco, M., Augugliaro, R., Giliani, S., Franceschini, R., Ochs, H. D., Wolf, H., Bonnefoy, J-Y., Biassoni, R., Moretta, L., Notarangelo, L. D., Moretta, A. (2000) X-linked lymphoproliferative disease: 2B4 molecules displaying inhibitory rather than activating func-

tion are responsible for the inability of natural killer cells to kill Epstein-Barr virus-infected cells. *J. Exp. Med.* **192,** 337–346.
127. Eissmann, P., Watzl, C. (2006) Molecular analysis of NTB-A signaling: a role for EAT-2 in NTB-A-mediated activation of human NK cells. *J. Immunol.* **177,** 3170–3177.
128. Ibegbu, C. C., Xu, Y-X., Harris, W., Maggio, D., Miller, J. D., Kourtis, A. P. (2005) Expression of killer cell lectin-like receptor G1 on antigen-specific human CD8+ T lymphocytes during active, latent, and resolved infection and its relation with CD57. *J. Immunol.* **174,** 6088–6094.
129. Ito, M., Ishida, T., He, L., Tanabe, F., Rongge, Y., Miyakawa, Y., Terunuma, H. (1998) HIV type 1 Tat protein inhibits interleukin 12 production by human peripheral blood mononuclear cells. *AIDS Res. Hum. Retroviruses* **14,** 845–849.
130. Wilson, C. C., Kalams, S., Wilkes, B., Ruhl, D., Gao, F., Hahn, B., Hanson, I., Luzuriaga, K., Wolinsky, S., Koup, R., Buchbinder, S., Johnson, R., Walker, B. (1997) Overlapping epitopes in human immunodeficiency virus type 1 gp120 presented by HLA A, B, and C molecules: effects of viral variation on cytotoxic T-lymphocyte recognition. *J. Virol.* **71,** 1256–1264.
131. Welte, S., Kuttruff, S., Waldhauer, I., Steinle, A. (2006) Mutual activation of natural killer cells and monocytes mediated by NKp80-AICL interaction. *Nat. Immunol.* **7,** 1334–1342.
132. Saudemont, A., Jouy, N., Hetuin, D., Quesnel, B. (2005) NK cells that are activated by CXCL10 can kill dormant tumor cells that resist CTL-mediated lysis and can express B7–H1 that stimulates T cells. *Blood* **105,** 2428–2435.
133. Trautmann, L., Janbazian, L., Chomont, N., Said, E. A., Gimmig, S., Bessette, B., Boulassel, M-R., Delwart, E., Sepulveda, H., Balderas, R. S., Routy, J-P., Haddad, E. K., Sekaly, R-P. (2006) Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. *Nat. Med.* **12,** 1198–1202.
134. Stern, N., Markel, G., Arnon, T. I., Gruda, R., Wong, H., Gray-Owen, S. D., Mandelboim, O. (2005) Carcinoembryonic antigen (CEA) inhibits NK killing via interaction with CEA-related cell adhesion molecule 1. *J. Immunol.* **174,** 6692–6701.
135. Berman, M. E., Xie, Y., Muller, W. (1996) Roles of platelet/endothelial cell adhesion molecule-1 (PECAM-1, CD31) in natural killer cell trans-endothelial migration and β 2 integrin activation. *J. Immunol.* **156,** 1515–1524.
136. Barber, D. F., Faure, M., Long, E. O. (2004) LFA-1 contributes an early signal for NK cell cytotoxicity. *J. Immunol.* **173,** 3653–3659.
137. Bryceson, Y. T., March, M. E., Ljunggren, H-G., Long, E. O. (2006) Activation, coactivation, and costimulation of resting human natural killer cells. *Immunol. Rev.* **214,** 73–91.
138. Pittet, M. J., Speiser, D. E., Valmori, D., Cerottini, J-C., Romero, P. (2000) Cutting edge: cytolytic effector function in human circulating CD8+ T cells closely correlates with CD56 surface expression. *J. Immunol.* **164,** 1148–1152.
139. Kärre, K., Ljunggren, H. G., Piontek, G., Kiessling, R. (1986) Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defense strategy. *Nature* **319,** 675–678.
140. Raulet, D. H., Vance, R. E., McMahon, C. W. (2001) Regulation of the natural killer cell repertoire. *Annu. Rev. Immunol.* **19,** 291–330.
141. Stulberg, M. J., Wright, P. W., Dang, H., Hanson, R. J., Miller, J. S., Anderson, S. K. (2006) Identification of distal KIR promoters and transcripts. *Genes Immun.* **8,** 124–130.
142. Hammond, S. M. (2005) Dicing and slicing: the core machinery of the RNA interference pathway. *FEBS Lett.* **579,** 5822–5829.
143. Davies, G. E., Locke, S. M., Wright, P. W., Li, H., Hanson, R. J., Miller, J. S., Anderson, S. K. (2007) Identification of bidirectional promoters in the human KIR genes. *Genes Immun.* **8,** 245–253.
144. Pascal, V., Stulberg, M. J., Anderson, S. K. (2006) Regulation of class I major histocompatibility complex receptor expression in natural killer cells: one promoter is not enough! *Immunol. Rev.* **214,** 9–21.
145. Vilches, C., Gardiner, C. M., Parham, P. (2000) Gene structure and promoter variation of expressed and nonexpressed variants of the KIR2DL5 gene. *J. Immunol.* **165,** 6416–6421.
146. Kubota, A., Kubota, S., Lohwasser, S., Mager, D. L., Takei, F. (1999) Diversity of NK cell receptor repertoire in adult and neonatal mice. *J. Immunol.* **163,** 212–216.
147. Uhrberg, M., Valiante, N. M., Shum, B. P., Shilling, H. G., Lienert-Weidenbach, K., Corliss, B., Tyan, D., Lanier, L. L., Parham, P. (1997) Human diversity in killer cell inhibitory receptor genes. *Immunity* **7,** 753–763.
148. Valiante, N. M., Uhrberg, M., Shilling, H. G., Lienert-Weidenbach, K., Arnett, K. L., D'Andrea, A., Phillips, J. H., Lanier, L. L., Parham, P. (1997) Functionally and structurally distinct NK cell receptor repertoires in the peripheral blood of two human donors. *Immunity* **7,** 739–751.
149. Yawata, M., Yawata, N., Draghi, M., Little, A-M., Partheniou, F., Parham, P. (2006) Roles for HLA and KIR polymorphisms in natural killer cell repertoire selection and modulation of effector function. *J. Exp. Med.* **203,** 633–645.
150. Shilling, H. G., Young, N., Guethlein, L. A., Cheng, N. W., Gardiner, C. M., Tyan, D., Parham, P. (2002) Genetic control of human NK cell repertoire. *J. Immunol.* **169,** 239–247.
151. Sivori, S., Cantoni, C., Parolini, S., Marcenaro, E., Conte, R., Moretta, L., Moretta, A. (2003) IL-21 induces both rapid maturation of human CD34+ cell precursors towards NK cells and acquisition of surface killer Ig-like receptors. *Eur. J. Immunol.* **33,** 3439–3447.
152. Bertone, S., Schiavetti, F., Bellomo, R., Vitale, C., Ponte, M., Moretta, L., Mingari, M. C. (1999) Transforming growth factor β-induced expression of CD94/NKG2A inhibitory receptors in human T lymphocytes. *Eur. J. Immunol.* **29,** 23–29.
153. Burgess, S. J., Marusina, A. I., Pathmanathan, I., Borrego, F., Coligan, J. E. (2006) IL-21 down-regulates NKG2D/DAP10 expression on human NK and CD8+ T cells. *J. Immunol.* **176,** 1490–1497.
154. Castriconi, R., Cantoni, C., Della Chiesa, M., Vitale, M., Marcenaro, E., Conte, R., Biassoni, R., Bottino, C., Moretta, L., Moretta, A. (2003) Transforming growth factor β 1 inhibits expression of NKp30 and NKG2D receptors: consequences for the NK-mediated killing of dendritic cells. *Proc. Natl. Acad. Sci. USA* **100,** 4120–4125.
155. Mingari, M. C., Vitale, C., Cantoni, C., Bellomo, R., Ponte, M., Schiavetti, F., Bertone, S., Moretta, A., Moretta, L. (1997) Interleukin-15-induced maturation of human natural killer cells from early thymic precursors: selective expression of CD94/NKG2-A as the only HLA class I-specific inhibitory receptor. *Eur. J. Immunol.* **27,** 1374–1380.
156. Mingari, M. C., Ponte, M., Bertone, S., Schiavetti, F., Vitale, C., Bellomo, R., Moretta, A., Moretta, L. (1998) HLA class I-specific inhibitory receptors in human T lymphocytes: interleukin 15-induced expression of CD94/NKG2A in superantigen- or alloantigen-activated CD8+ T cells. *Proc. Natl. Acad. Sci. USA* **95,** 1172–1177.
157. Tajima, K., Matsumoto, N., Ohmori, K., Wada, H., Ito, M., Suzuki, K., Yamamoto, K. (2004) Augmentation of NK cell-mediated cytotoxicity to tumor cells by inhibitory NK cell receptor blockers. *Int. Immunol.* **16,** 385–393.
158. Fernandez, N. C., Treiner, E., Vance, R. E., Jamieson, A. M., Lemieux, S., Raulet, D. H. (2005) A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules. *Blood* **105,** 4416–4423.
159. Kim, S., Poursine-Laurent, J., Truscott, S. M., Lybarger, L., Song, Y-J., Yang, L., French, A. R., Sunwoo, J. B., Lemieux, S., Hansen, T. H., Yokoyama, W. M. (2005) Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature* **436,** 709–713.
160. Yokoyama, W. M., Kim, S. (2006) How do natural killer cells find self to achieve tolerance? *Immunity* **24,** 249–257.
161. Gasser, S., Raulet, D. H. (2006) Activation and self-tolerance of natural killer cells. *Immunol. Rev.* **214,** 130–142.
162. Oppenheim, D. E., Roberts, S. J., Clarke, S. L., Filler, R., Lewis, J. M., Tigelaar, R. E., Girardi, M., Hayday, A. C. (2005) Sustained localized expression of ligand for the activating NKG2D receptor impairs natural cytotoxicity in vivo and reduces tumor immunosurveillance. *Nat. Immunol.* **6,** 928–937.
163. Parham, P. (2005) Immunogenetics of killer cell immunoglobulin-like receptors. *Mol. Immunol.* **42,** 459–462.
164. Yawata, M., Yawata, N., Abi-Rached, L., Parham, P. (2002) Variation within the human killer cell immunoglobulin-like receptor (KIR) gene family. *Crit. Rev. Immunol.* **22,** 463–482.
165. Maxwell, L. D., Wallace, A., Middleton, D., Curran, M. D. (2002) A common KIR2DS4 deletion variant in the human that predicts a soluble KIR molecule analogous to the KIR1D molecule observed in the rhesus monkey. *Tissue Antigens* **60,** 254–258.
166. Yawata, M., Yawata, N., McQueen, K., Cheng, N., Guethlein, L., Rajalingam, R., Shilling, H., Parham, P. (2002) Predominance of group A KIR haplotypes in Japanese associated with diverse NK cell repertoires of KIR expression. *Immunogenetics* **54,** 543–550.
167. Hiby, S. E., Walker, J. J., O'Shaughnessy, K. M., Redman, C. W. G., Carrington, M., Trowsdale, J., Moffett, A. (2004) Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *J. Exp. Med.* **200,** 957–965.
168. Du, Z., Gjertson, D., Reed, E., Rajalingam, R. (2007) Receptor-ligand analyses define minimal killer cell Ig-like receptor (KIR) in humans. *Immunogenetics* **59,** 1–15.
169. van der Slik, A. R., Alizadeh, B., Koeleman, B., Roep, B., Giphart, M. (2007) Modelling KIR-HLA genotype disparities in type 1 diabetes. *Tissue Antigens* **69,** 101–105.

170. van der Slik, A. R., Koeleman, B. P. C., Verduijn, W., Bruining, G. J., Roep, B. O., Giphart, M. J. (2003) KIR in type 1 diabetes: disparate distribution of activating and inhibitory natural killer cell receptors in patients versus HLA-matched control subjects. *Diabetes* **52,** 2639–2642.
171. López-Larrea, C., Njobvu, P. D., González, S., Blanco-Gelaz, M. A., Martínez-Borra, J., López-Vázquez, A. (2005) The HLA-B*5703 allele confers susceptibility to the development of spondylarthropathies in Zambian human immunodeficiency virus-infected patients with slow progression to acquired immunodeficiency syndrome. *Arthritis Rheum.* **52,** 275–279.
172. Altfeld, M., Kalife, E. T., Qi, Y., Streeck, H., Lichterfeld, M., Johnston, M. N., Burgett, N., Swartz, M. E., Yang, A., Alter, G., Yu, X. G., Meier, A., Rockstroh, J. K., Allen, T. M., Jessen, H., Rosenberg, E. S., Carrington, M., Walker, B. D. (2006) HLA alleles associated with delayed progression to AIDS contribute strongly to the initial CD8+ T cell response against HIV-1. *PLoS Med.* **3,** e403.
173. Carrington, M., O'Brien, S. J. (2003) The influence of HLA genotype on AIDS. *Annu. Rev. Med.* **54,** 535–551.
174. Heeney, J. L., Plotkin, S. A. (2006) Immunological correlates of protection from HIV infection and disease. *Nat. Immunol.* **7,** 1281–1284.
175. McMichael, A. J., Rowland-Jones, S. L. (2001) Cellular immune responses to HIV. *Nature* **410,** 980–987.
176. Flores-Villanueva, P. O., Yunis, E. J., Delgado, J. C., Vittinghoff, E., Buchbinder, S., Leung, J. Y., Uglialoro, A. M., Clavijo, O. P., Rosenberg, E. S., Kalams, S. A., Braun, J. D., Boswell, S. L., Walker, B. D., Goldfeld, A. E. (2001) Control of HIV-1 viremia and protection from AIDS are associated with HLA-Bw4 homozygosity. *Proc. Natl. Acad. Sci. USA* **98,** 5140–5145.
177. Qi, Y., Martin, M. P., Gao, X., Jacobson, L., Goedert, J. J., Buchbinder, S., Kirk, G. D., Brien, S. J., Trowsdale, J., Carrington, M. (2006) KIR/HLA pleiotropism: protection against both HIV and opportunistic infections. *PLoS Pathog.* **2,** e79.
178. Gaudieri, S., DeSantis, D., McKinnon, E., Moore, C., Nolan, D., Witt, C. S., Mallal, S. A., Christiansen, F. T. (2005) Killer immunoglobulin-like receptors and HLA act both independently and synergistically to modify HIV disease progression. *Genes Immun.* **6,** 683–690.
179. Gaudieri, S., Nolan, D., McKinnon, E., Witt, C. S., Mallal, S., Christiansen, F. T. (2005) Associations between KIR epitope combinations expressed by HLA-B/-C haplotypes found in an HIV-1 infected study population may influence NK mediated immune responses. *Mol. Immunol.* **42,** 557–560.
180. Lopez-Vazquez, A., Mina-Blanco, A., Martinez-Borra, J., Njobvu, P. D., Suarez-Alvarez, B., Blanco-Gelaz, M. A., Gonzalez, S., Rodrigo, L., Lopez-Larrea, C. (2005) Interaction between KIR3DL1 and HLA-B*57 supertype alleles influences the progression of HIV-1 infection in a Zambian population. *Hum. Immunol.* **66,** 285–289.
181. Martin, M., Pascal, V., Yeager, M., Phair, J., Kirk, G., Hoots, K., O'Brien, S., Anderson, S., Carrington, S. (2007) A mutation in KIR3DS1 that results in truncation and lack of cell surface expression. *Immunogenetics* **59,** 823–829.
182. Pascal, V., Yamada, E., Martin, M. P., Alter, G., Altfeld, M., Metcalf, J. A., Baseler, M. W., Adelsberger, J. W., Carrington, M., Anderson, S. K., McVicar, D. W. (2007) Detection of KIR3DS1 on the cell surface of peripheral blood NK cells facilitates identification of a novel null allele and assessment of KIR3DS1 expression during HIV-1 infection. *J. Immunol.* **179,** 1625–1633.
183. Jennes, W., Verheyden, S., Demanet, C., Adje-Toure, C. A., Vuylsteke, B., Nkengasong, J. N., Kestens, L. (2006) Cutting edge: resistance to HIV-1 infection among African female sex workers is associated with inhibitory KIR in the absence of their HLA ligands. *J. Immunol.* **177,** 6588–6592.
184. Zhou, H., Luo, Y., Lo, J-f., Kaplan, C. D., Mizutani, M., Mizutani, N., Lee, J-D., Primus, F. J., Becker, J. C., Xiang, R., Reisfeld, R. A. (2005) DNA-based vaccines activate innate and adaptive antitumor immunity by engaging the NKG2D receptor. *Proc. Natl. Acad. Sci. USA* **102,** 10846–10851.
185. Ravet, S., Scott-Algara, D., Bonnet, E., Tran, H. K., Tran, T., Nguyen, N., Truong, L. X., Theodorou, I., Barre-Sinoussi, F., Pancino, G., Paul, P. (2007) Distinctive NK-cell receptor repertoires sustain high-level constitutive NK-cell activation in HIV-exposed uninfected individuals. *Blood* **109,** 4296–4305.
186. Scott-Algara, D., Truong, L. X., Versmisse, P., David, A., Luong, T. T., Nguyen, N. V., Theodorou, I., Barre-Sinoussi, F., Pancino, G. (2003) Cutting edge: increased NK cell activity in HIV-1-exposed but uninfected Vietnamese intravascular drug users. *J. Immunol.* **171,** 5663–5667.
187. Barbour, J. D., Sriram, U., Caillier, S. J., Levy, J. A., Hecht, F. M., Oksenberg, J. R. (2007) Synergy or independence? Deciphering the interaction of HLA class I and NK cell KIR alleles in early HIV-1 disease progression. *PLoS Pathog.* **3,** e43.
188. Alter, G., Martin, M. P., Teigen, N., Carr, W. H., Suscovich, T. J., Schneidewind, A., Streeck, H., Waring, M., Meier, A., Brander, C., Lifson, J. D., Allen, T. M., Carrington, M., Altfeld, M. (2007) Differential natural killer cell mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes. *J. Exp. Med.* **204,** 3027–3036.
189. Maenaka, K., Juji, T., Nakayama, T., Wyer, J. R., Gao, G. F., Maenaka, T., Zaccai, N. R., Kikuchi, A., Yabe, T., Tokunaga, K., Tadokoro, K., Stuart, D. I., Jones, E. Y., van der Merwe, P. A. (1999) Killer cell immunoglobulin receptors and T cell receptors bind peptide-major histocompatibility complex class I with distinct thermodynamic and kinetic properties. *J. Biol. Chem.* **274,** 28329–28334.
190. Sawicki, M. W., Dimasi, N., Natarajan, K., Wang, J., Margulies, D. H., Mariuzza, R. A. (2001) Structural basis of MHC class I recognition by natural killer cell receptors. *Immunol. Rev.* **181,** 52–65.
191. Trundley, A., Frebel, H., Jones, D., Chang, C., Trowsdale, J. (2007) Allelic expression patterns of KIR3DS1 and 3DL1 using the Z27 and DX9 antibodies. *Eur. J. Immunol.* **37,** 780–787.
192. Matte, C., Lajoie, J., Lacaille, J., Zijenah, L., Ward, B., Roger, M. (2004) Functionally active HLA-G polymorphisms are associated with the risk of heterosexual HIV-1 infection in African women. *AIDS* **18,** 427–431.
193. Lajoie, J., Hargrove, J., Zijenah, L. S., Humphrey, J. H., Ward, B. J., Roger, M. (2006) Genetic variants in nonclassical major histocompatibility complex class I human leukocyte antigen (HLA)-E and HLA-G molecules are associated with susceptibility to heterosexual acquisition of HIV-1. *J. Infect. Dis.* **193,** 298–301.
194. Boulet, S., Sharafi, S., Simic, N., Bruneau, J., Routy, J. P., Tsoukas, C. M., Bernard, N. F. (2008) Increased proportion of KIR3DS1 homozygotes in HIV-exposed uninfected individuals. *AIDS* **22,** 595–599.

## 5- Les cellules Natural Killer et l'infection par le VIH-1: conséquences sur l'élaboration de stratégies thérapeutiques et la vaccination.

Au cours de la dernière décennie, de considérables progrès ont été réalisés dans la compréhension de l'interaction du VIH-1 avec les cellules NK. Cet article de revue traite de la réponse antivirale médiée par ces cellules, des stratégies élaborées par le VIH-1 pour leur échapper, et de l'importance de ces cellules dans le contrôle de l'infection et du développement du SIDA. Cette revue met aussi en relation l'immunobiologie des cellules NK sur laquelle se base leur possible utilisation en tant qu'agents thérapeutiques en consignant les limites et les problèmes auxquels les scientifiques seront exposés. Cet article a été soumis à un processus de révision par les pairs avant sa publication.

**Article 5 :** **Iannello A**, Debbeche O, Samarani S, and Ahmad A. Antiviral NK Cell Responses in HIV Infection: II. Viral strategies for evasion and lessons for Immunotherapy and Vaccination. 2008. ***Journal of Leukocyte Biology*** **84(1): 27-49**.

**Résumé :** Comme c'est le cas dans d'autres infections virales, le système immunitaire humain répond à l'infection par le VIH-1 en activant ces cellules NK. Le VIH-1 a élaboré plusieurs stratégies pour échapper à la réponse antivirale médiée par ces cellules. Par conséquent, les fonctions des cellules NK sont compromises chez les patients infectés et ce très tôt lors de l'infection, ce qui engendrera indirectement un défaut dans la réponse acquise de ces patients. Des progrès importants ont été réalisés dans la compréhension des mécanismes d'évasions élaborés par le VIH-1 pour contrer la réponse antivirale médiée par les cellules NK. La connaissance de ces mécanismes a permis de proposer et d'élaborer de nouvelles stratégies thérapeutiques et vaccinales contre ce virus. Cette revue décrit comment la réponse médiée par les cellules NK a lieu lors de l'infection et documente les stratégies d'évasions développées par le VIH-1 pour contrer cette réponse. Cet article met ausi en relation ces phénomènes et leurs implications sur ce qui pourrait amener la communauté scientifique à l'élaboration de nouvelles approches thérapeutiques.

# Article 5

**Iannello A**, Debbeche O, Samarani S, and Ahmad A. Antiviral NK Cell Responses in HIV Infection: II. Viral strategies for evasion and lessons for Immunotherapy and Vaccination. 2008. ***Journal of Leukocyte Biology*** **84(1): 27-49**.

# Antiviral NK cell responses in HIV infection: II. viral strategies for evasion and lessons for immunotherapy and vaccination

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**Abstract: As is the case in other viral infections, humans respond to HIV infection by activating their NK cells. However, the virus uses several strategies to neutralize and evade the host's NK cell responses. Consequently, it is not surprising that NK cell functions become compromised in HIV-infected individuals in early stages of the infection. The compromised NK cell functions also adversely affect several aspects of the host's antiviral adaptive immune responses. Researchers have made significant progress in understanding how HIV counters NK cell responses of the host. This knowledge has opened new avenues for immunotherapy and vaccination against this infection. In the first part of this review article, we gave an overview of our current knowledge of NK cell biology and discussed how the genes encoding NK cell receptors and their ligands determine innate genetic resistance/susceptibilty of humans against HIV infections and AIDS. In this second part, we discuss NK cell responses, viral strategies to counter these responses, and finally, their implications for anti-HIV immunotherapy and vaccination. *J. Leukoc. Biol.* 84: 27–49; 2008.**

***Key Words:*** *ADCC · AIDS · CD94/NKG2 · chemokines · cytokines · HIV-1 · HLA · KIR · KIR haplotypes · MHC class I · MICA · MICB · NK cell receptors · NKG2D · ULBP*

## INTRODUCTION

This is second part of a review article on NK cell responses in HIV infections. The first part gives an overview of our current knowledge about NK cell immunobiology, receptors, and their ligands. The part also describes how polymorphism in the genes encoding killer-cell Ig-like receptor (KIR) and their HLA ligands determines innate genetic resistance/susceptibility to HIV infection and development of AIDS. This second part of the article deals with functional defects that occur in NK cells in the course of HIV infection, viral strategies to counter host's NK cell responses, and their implications for anti-HIV immunotherapy and vaccination. We recommend that this article be read in conjunction with its first part.

## NK CELL ACTIVATION IN HIV INFECTION

As mentioned earlier, NK cells are present in the circulation, bone marrow, lymph nodes, spleen, lung, liver, omentum, etc., and can reach almost any place in the body where a viral infection occurs and induces an inflammatory response. Viral infections generally activate NK cells, especially in early stages of the infection. Studies from animal models as well as in individuals in acute stages of the infection suggest that HIV is no exception to this rule. The infection also causes activation and expansion of NK cells. NK cell activation and expansion have been observed in humans in primary HIV infections and precede the appearance of virus-specific CTL responses. The expansion usually occurs in the highly cytotoxic $CD56^{dim}CD16^{+}$ subset of NK cells [1]. Increased NK cell activities were also observed in monkeys after experimental infection with SIV [2]. This initial NK cell expansion and activation probably result from direct and indirect effects of the infection. Virus-induced cytokines, e.g., type I IFN, IL-12, IL-15, IL-18, etc., are usually responsible for early NK cell activation and expansion. Viral proteins and nucleic acids may bind to TLRs and/or other receptors on a variety of host cells including NK cells themselves, resulting in their activation. As mentioned in the first part of this review, to become functionally competent, TLRs expressed on NK cells seem to require help from accessory cells [3, 4]. Thus, it is not surprising that a uridine-rich ssRNA derived from HIV-1 long-terminal repeat has been shown to activate NK cells but requires the presence and activation of plasmacytoid DC or $CD14^{+}$ monocytes [5]. Activated NK cells activate DC, secrete IFN-γ, and act as adjuvants by killing virus-infected cells and by causing release of intracellular proteins from the killed cells. NK cell activation has been shown to be important in inducing an effective adaptive immune response against intracellular pathogens in several animal models. In the context of HIV infection, NK cells may control the infection, not only by killing virus-infected cells directly as well as indirectly by antibody-dependent, cell-mediated cytotoxicity (ADCC), but also, they serve as an important source of β-chemokines (MIP-1α, MIP-1β, and RANTES) and undefined soluble factors, which can sup-

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press replication of M- and T-tropic HIV viruses [6–8]. NK cell-secreted cytokines, especially IFNs, may induce the antiviral state in host cells and cure HIV-infected cells via noncytolytic mechanisms.

Many studies have shown that NK cells play an important role in controlling HIV replication. The presence of NK cells suppresses HIV replication in cell cultures [9–11]. It has been demonstrated that i.v. drug users, who are at high risk of contracting HIV infection, resist infection, as long as they have elevated NK cell activities. In this regard, researchers have shown that these uninfected but highly exposed drug users have NK cells, which produce more chemokines and cytokines in vitro with or without simulation and whose KIR repertoire is predominantly of an activating type. They have high ratios of KIR3DS1$^+$/KIR3DL1$^+$ and NK cell group 2C+ (NKG2C$^+$)/NKG2A$^+$ NK cells and coinherit the weakly inhibiting *KIR-MHC* gene pair (*KIR2DL3/HLA-C* of group I). They also have low expression of KIR3DL1 and an increased expression of CD107a and CD69 on their NK cells [12, 13]. Apart from protecting from HIV infection, high NK cell activities also delay progression of the infection toward AIDS [14, 15]. It has been demonstrated that decreases in NK cell cytotoxicity as well as in NK cell counts in the circulation of the infected persons were associated with their rapid CD4$^+$ T cell depletions and rapid progression toward AIDS [14, 16]. However, the infected persons who are able to maintain their NK cell functions remain healthy, despite having decreased CD4$^+$ T cell counts [15]. Animal models of HIV infection also support a role of NK cells in controlling this infection. It has been shown that our closest relatives, chimpanzees (Pan troglydytes), can be infected with HIV-1 and SIV$_{cpz}$. The viruses replicate in this species but cause no AIDS-like disease. It is noteworthy that NK cells are more abundant in this species than in humans; they remain fully functional throughout the course of infection and unlike humans, can up-regulate certain natural cytotoxicity receptors (NCR; NKp30) in response to the infection. As mentioned above, this receptor plays a role in NK cell–dendritic cell (DC) interactions. Higher NK cell responses in chimpanzees are thought to be a factor in their resistance to progression to an AIDS-like disease [17, 18].

Several workers have investigated NK cell responses in HIV-infected humans. An exhaustive list of these studies, along with their major findings, is given in **Table 1**. It is quite evident from this table that NK cell functions (cytolytic and secretory) become compromised in HIV-infected persons; depletion of functional NK cell subsets and expansion of nonfunctional NK cells occurs; the infection causes changes in the expression of NKRs and their ligands; HAART tends to normalize changes in the number and functional capabilities of NK cells, but they never become normal. Few studies have been undertaken to translate our current knowledge into ways and means to invigorate NK cells and develop novel, anti-HIV vaccines.

## ADCC

NK cells not only can kill virus-infected cells alone, they can also do so in combination with antibodies for which the antigen is expressed on the surface of the infected cells. The process is called ADCC. The antibodies bind through their variable antigen-binding sites to the viral antigen on the surface of the virus-infected cells and through their so-called crystallizable fragments (Fc) to CD16 on NK cells [63, 64]. The antibodies cross-link CD16 on NK cells and consequently, trigger their cytolytic functions. This results in killing of the virus-infected cells and secretion of cytokines and chemokines from NK cells (**Fig. 1**). The ADCC is a classical example of cooperation between innate and adaptive immune responses in protecting host from viral infections and malignancies. CD16 is a type I Ig-like integral membrane glycoprotein, which is expressed on the surface of NK cells, monocyte-macrophages, Langerhan's cells, DC, etc. It is a low-affinity type III receptor for the Fc part of IgG (FcγRIII; CD16). It binds aggregated but not monomeric human IgG1 and IgG3. The aggregated Ig are present in immune complexes. NK cells express the CD16A or FcγRIIIA form of the receptor. This form associates noncovalently via its transmembrane region with signaling adaptors (γ and/or ζ chains) and can transmit signals intracellularly. The receptor plays a predominant role in NK cell-mediated ADCC. Therefore, it is also commonly referred to as the "ADCC receptor." Another form of the receptor (CD16B or FcγRIIIB) is anchored in the plasma membrane via GPI and cannot transmit intracellular signals. This form acts as a sink for antigen/antibody complexes and is expressed on neutrophils and eosinophils [64]. The level of expression of CD16 on the surface of NK cells correlates with their functional ADCC activity. CD16 interacts physically with CD38 on the surface of NK cells. CD38 is a surface glycoprotein with ADP ribosyl cyclase/cyclic ADP-ribose hydrolase activities. It regulates cytoplasmic calcium and also acts as a receptor modulating cell–cell interactions. It binds CD31 (PECAM-1), which is a transmembrane Ig-like glycoprotein expressed on human vascular endothelial cells, and plays a role in angiogenesis, cell adhesion, and diapedesis. When cross-linked, CD38 transmits activating signals to NK cells via CD16 [65]. Interestingly, cells may shed CD16 upon activation, and cleaved sCD16 interferes with the ADCC process. Increased concentrations of sCD16 have been reported in the sera of HIV-infected persons, which correlate with disease progression. Interestingly, sCD16 seems to be shed from non-NK cells in these patients [66].

In addition to CD16, NK cells express an activating version of the FcγRIIC (CD32C), which also takes part in ADCC [67]. However, only 40–45% humans express this receptor on their NK cells. It is noteworthy that CD32 is encoded by three diferent genes: *CD32A*, *-B*, and *-C*. CD32A is an activating receptor expressed on neutrophils, monocytes, and DC. CD32B is an inhibitory receptor expressed on DC, monocytes, neutrophils, and B cells. An allelic variant of CD32A expresses arginine at position 131 (R131) instead of histidine (H131). The R131 variant responds vigorously to IgG and has been implicated in the development of systemic lupus erythematosus (for details, see ref. [67]).

The major FcR involved in ADCC may be mutated and nonfunctional in some individuals. This happens as a result of a deletion of a single base (adenine) in exon 4 at nucleotide 550, resulting in a premature stop codon and truncated protein [68, 69]. Another mutation has been described that results in

polymorphism at position 158 in the amino-acid sequence. The amino acid at this position could be valine (V) or phenylalanine (F). The V allotype has higher affinity with IgG than the F one. The individuals with the *V/V* genotype are more efficient in mediating ADCC [70]. Few studies have investigated the impact of these mutations on the clinical course of HIV infection. In this regard, one group of researchers has demonstrated that HIV-infected persons bearing the FcγRII RR genotype progress more rapidly toward AIDS than those bearing HH or HR genotypes [71].

The ADCC-mediated destruction of tumor cells as well as of virus-infected cells can be readily demonstrated in vitro in the presence of NK cells and tumor or virus-specific antibodies of the appropriate IgG isotypes. The process also occurs in vivo. Although macrophages and neutrophils can also mediate ADCC, NK cells are the main cell type that mediates this process. Their depletion, therefore, abrogates the ADCC-mediating ability of PBMC [64].

## ADCC IN HIV INFECTION

NK cells may eliminate HIV-infected cells in combination with HIV-specific antibodies via ADCC. The destruction of HIV-infected cells can be readily demonstrated in in vitro ADCC reactions in which HIV-infected cells are incubated with NK cells in the presence of HIV-specific antibodies. The phenomenon has been demonstrated to occur in vivo in these infections [26]. The antibodies specific to the viral envelope protein gp120/41 have been shown to mediate ADCC against the virus-infected cells. A prerequisite of the ADCC against HIV-infected cells is that the virus must be replicating in the cells, and the viral envelope proteins must be expressed on the surface of these cells. Anti-HIV, ADCC-mediating antibodies have been demonstrated in the sera of HIV-infected persons in several studies [9, 33, 63].

Although HIV-specific ADCC eliminates HIV-infected cells, it also has the potential to contribute to AIDS pathogenesis (Fig. 1). In in vitro experiments, uninfected $CD4^+$ T cells may bind exogenous recombinant gp120 and be killed by NK cells in the presence of gp120-specific antibodies of the IgG isotype [72, 73]. Furthermore, anti-gp120 antibodies may complex with the virus and facilitate uptake of the virus by monocytes. They may also cause NK cell activation and hence, excessive production of chemokines and cytokines. In fact, a group has demonstrated a correlation between the presence of HIV-specific ADCC antibodies and the development of AIDS [74]. However, these studies have not been corroborated. On the other hand, several researchers have demonstrated that these antibodies correlate with better clinical condition and better prognosis in HIV-infected children and adults [63, 75–77]. The protective nature of anti-HIV ADCC antibodies could also be demonstrated in in vitro experiments in which HIV-specific antibodies or NK cells alone are not able to inhibit replication of primary isolates of HIV-1 in human PBMC. However, they do so efficiently via ADCC when added together to these cultures [9]. Studies in animal models of HIV infection have also shown a protective effect of ADCC against disease progression [78]. Many researchers regard anti-HIV ADCC as a reliable correlate of immune protection from HIV infection [17, 63]. However, it remains to be tested in HIV vaccination studies. It has been demonstrated that vaccines may elicit ADCC antibodies, which could inhibit replication of clinical strains of HIV in the presence of NK cells [9].

Although anti-HIV ADCC antibodies can be demonstrated in HIV-infected individuals, even in late stages of the infection, the full host beneficial potential of this ADCC cannot be realized in vivo, as NK cell functions become compromised in a majority of these individuals [14, 27, 79, 80]. The decreased ADCC effector function of NK cells in HIV-infected persons could be a result of several reasons: decreased number of $CD16^+$ NK cells, decreased expression of the signaling partner ζ chain in NK cells, and overall decreased cytolytic capacity of NK cells (see Table 1). It is noteworthy that the engagement of CD16 alone cannot mediate killing of the target cells. For this purpose, it needs simultaneous engagement of LFA-1 or 2B4 (reviewed in ref. [81]). The receptor activities may be neutralized by increased concentrations of ICAMs and sCD16 in the circulation of HIV-infected patients [66, 82]. Interestingly, these concentrations increase with disease progression and serve as prognostic markers. Increased expression of HLA-C and -E on the surface of HIV-infected T cell blasts also interferes with their killing by autologous NK cells via ADCC. The blockage of interactions between KIR and HLA-C and between NKG2A and HLA-E with specific antibodies enhances this immune effector mechanism against this virus [83].

Attempts to control HIV replication in HIV-infected patients via passive immunotherapy (infusion of anti-HIV antibodies or i.v. Igs) have not yielded desired results. Passively infused i.v. Igs are known to have immunosuppressive effects (reviewed in ref. [84]). The infused antibodies form multimeric IgG complexes on DC. Such DC are killed by NK cells via ADCC or become defective for their ability to activate NK cells and to prime T cells. They decrease the expression of NKp30 and KIR on interacting NK cells [85]. Therefore, they may aggravate the defects, which already exist in an NK cell compartment in HIV-infected patients. HIV-1 has developed myriad strategies to evade a neutralizing antibody response of the host, e.g., mutation of epitopes, masking of epitopes by glycosylation and trimerization of gp120/41 spikes, shedding of envelope proteins, etc. (reviewed in ref. [86]). Nevertheless, the infusion of a combination of HIV-specific neutralizing antibodies does provide protection from infection in the animal models. However, it has been demonstrated that the antibodies require binding to FcγR for full efficacy [87, 88]. These results highlight a beneficial role of ADCC for the host. FcγR can also mediate uptake of antibody-coated viruses by monocytes and macrophages. The potentials and limitations of the i.v. use of neutralizing antibodies in HIV-infected patients have been demonstrated by the results of a small trial, in which a combination of HIV-neutralizing antibodies was infused into HIV-infected persons. Their HAART treatment was stopped 1 day after the infusion, and HIV rebound was measured [89, 90]. The virus rebound was delayed in acutely infected persons. However, this delay in the virus rebound was seen only in two of the chronically infected persons. Escape mutants also appeared for one of the three antibodies in the rebound viruses.

TABLE 1. List of Published Research Papers and Their Major Findings on NK Cells in HIV-Infected Persons

| Year | Major Findings | Reference |
|---|---|---|
| *1986* | Defective trigger and release of NKCF; partial restoration by IL-2. | [19] |
| *1986* | Decreased NK cell activity as a result of decreased IL-2 production; *in vitro* IL-2 restores this activity. | [20] |
| *1986* | IL-2 increases NK cell-mediated killing of HIV-1-infected cells; HIV-1 infects NK cells *in vitro.* | [21] |
| *1987* | Defective natural cytotoxicity, normal ADCC. | [22] |
| *1987* | Defective NK cell lysis in patients with AIDS and AIDS-related complex; increased number of $CD8^+CD57^+$ and $CD16^+CD57^+$ cells. | [23] |
| *1988* | Normal actin polarization but defective tubulin rearrangement in NK cells from AIDS patients. | [24] |
| *1990* | Defective inducibility of LAK activity in blood lymphocytes. | [25] |
| *1990* | Defective ADCC effector functions of NK cells. | [26] |
| *1990* | CD16+ NK cells decrease in all stages of infection; specific depletion of $CD3^-CD8^+CD16^+$ cells. | [27] |
| *1992* | NK cell activation and killing decreased, not restored by long-term in vitro culture with IL-2; defect at clonal level. | [28] |
| *1994* | $CD56^+$ and $KIR^+$ NK cells decrease in AIDS patients; NK cell activity decreases, and $EB6^+$ $GL183^+$ (double-positive) NK and T cells appear in all HIV-infected persons. | [29] |
| *1994* | Impaired NK and LAK activity; no effect of AZT therapy. | [30] |
| *1994* | NK cell activity decreased with disease progression starting in CDC stage IVC2; Defect not corrected by in vitro culture with IL-2. | [31] |
| *1994* | Decreased $CD16^+CD56^+$ NK cells in primary and chronic HIV infections in children. | [32] |
| *1994* | Defective ADCC effector function of NK cells from HIV-infected persons; IL-2 and IFN-$\gamma$ restore partially. | [33] |
| *1995* | Decreased NK cell activity early in infection; CD16+ decreased; $CD16^+CD56^+$ percentage normal or elevated, reduced response to IFN-$\alpha$. | [34] |
| *1995* | $CD16^+CD56^+$ subset decreases; expansion of less-cytotoxic $CD16^{dim}CD56^-$ cells. | [35] |
| *1995* | $CD8^+CD16^+CD56^+$ NK cells depleted. | [36] |
| *1996* | Defective NK cell-mediated killing of gp120/41-expressing target cells. | [37] |
| *1997* | $CD16^+$, $CD56^+$ and $CD16^+CD56^+$ NK cell percentage and concentration of $CD16^+CD56^+$ NK cells decrease in correlation with disease progression. | [38] |
| *1997* | AIDS patients have lower NK cell activity than HIV-infected asymptomatic and seronegative controls; at least in part as a result of reduced IL-2 production. | [39] |
| *1997* | $KIR^+$ $CD3^+$ $CD8^+$ cells increase, masking KIR-increased cytotoxicity against HIV-infected cells. | [40] |
| *1999* | Increased percentage of $CD94^+CD8^+CD56^+$ cells; no change in KIR, IL-10 implicated. | [41] |
| *1999* | KIR increased transiently on T but not on NK cells; CD94 increases on both cell types. | [42] |
| *1999* | Poor response to IFN-$\alpha$ and low LAK activity associated with poor prognosis and death. | [43] |
| *1999* | Decreased expression of $\zeta$ chain in NK cells; defective, CD16-mediated lysis. | [44] |
| *2001* | NK activity decreased with decrease in VL; decreased iKIR on $CD56^+$ but increased on $CD8^+$ cells; CD57 and CD94 expression increased. | [16] |
| *2001* | Decreased NK cell numbers with decrease in $CD4^+$ T cell counts; decreased $CD16^+CD56^+$ NK cells; expansion of $CD8^+CD38^+DR^+$ lymphocytes associated with low cytotoxicity. | [14] |
| *2001* | HAART normalizes expression of iKIR and NKG2A but not of ILT-2 on CTL; blocking of ILT-2 increases CTL activity. | [45] |
| *2002* | $CD56^+$ cells coexpressing CD16, CD161, or CD94 become depleted; CD56– cells coexpressing CD16, CD161, or CD94 expand; defective differentiation. | [46] |
| *2002* | Defective modulation of CD69 and CD16 expression on NK cells. | [47] |
| *2002* | Increased NK cell activity that decreases with HAART; more NK cells expressing CD158a and CD94 appear; IL-10 implicated. | [48] |
| *2003* | Decreased production of chemokines and viral suppression by NK cells from viremic patients. | [11] |
| *2003* | Decreased expression of NCR on fresh and in vitro-activated NK cells. | [49] |
| *2003* | Decrease in CD16–$CD56^{bright}$ subset in patients undergoing HAART; IL-12 plus IL-15 or HGH partially restore NK cell number and IFN-$\gamma$ production. | [50] |
| *2003* | Expansion of $CD56^-CD16^+$ dysfunctional NK subset; decreased expression of actNKR but not of NKG2D; stable or increased KIR expression; impaired NK cell cytotoxicity; HAART normalizes NKR expression. | [51] |
| *2004* | Aberrant NK cell activation; NCR expression decreased; no NKp44 expression; decreased cytotoxicity in viremic patients. | [52] |
| *2004* | Increased CCR5 and iKIR expression on NK in viremia; became normal after HAART; direct correlation of iKIR expression with viral load. | [7] |
| *2004* | iKIR expression increases on NK in viremic patients; $CD56^+CD94^+$ and $CD56^+CD161^+$ NK decreased; NK cell number decreased; overall NK activity and CD107a expression increased; NK produce more TNF-$\alpha$ and IFN-$\gamma$ in viremic patients. | [1] |
| *2005* | Expansion of $NKG2C^+$ and loss of $NKG2A^+$ NK; no effect of HAART. | [53] |
| *2005* | Expansion of dysfunctional $CD56^-CD16^+$ subset in viremia; iKIR expression increased on NK; major activating NKR decreased but not NKG2D; NK less cytotoxic, produce less chemokines and cytokines. | [54] |
| *2005* | Decreased $CD94^+$ NK cell numbers in viremic patients and advanced disease. | [55] |
| *2005* | $2B4^+$ cells transiently decrease early in infection; decrease is related to VL and sTNFRII; later expression increases on NK and T cells and normalizes with HAART. | [56] |
| *2005* | Increased NK cell numbers with early depletion of the $CD56highCD16^-$ subset and expansion of the $CD56lowCD16^+$ subset in acute infection; the depletion of the $CD56^{low}CD16^+$ subset continues with a parallel increase in the anergic $CD56^-CD16^+$ subset. | [57] |
| *2006* | Decreased perforin and increased SHIP expression in NK cells in chronic infection. | [58] |
| *2007* | Expression of inhibitory FcRL6 on all lymphocytes (NK, $CD4^+$, $CD8^+$ effector memory cells). | [59] |

TABLE 1. (Continued)

| Year | Major Findings | Reference |
|---|---|---|
| 2007 | NK cell activation and expansion precede CTL response in acute infection; NK cell activity decreases with HAART; NK and CTL respond with similar kinetics upon antigen exposure or upon discontinuation of therapy. | [60] |
| 2007 | CD56dim NK cell subset expanded in primary infection; partial normalization after 6 months HAART; CD27$^+$CD70$^+$ expression increased in chronic infection. | [61] |
| 2007 | KIR3DS1$^+$ NK and T cells increase in number in viremic HIV-infected persons. | [62] |

actNKR, Activating NK cell receptors (NKR); FcRL6, FcR-like protein 6; HGH, human growth hormone; iKIR, inhibitory KIR; LAK, lymphokine-activated killer cell activity; NKCF, NK cell cytotoxic factor, HAART, highly active antiviral therapy; sTNFRII, soluble type II TNFR; VL, viral load; ILT-2, Ig-like transcript 2. EB6 and GL183 are mouse MAb (from Immunotech/Coulter, France) that bind to KIR2DL1 (p58.1) and KIR2DL2/3 (p58.2), respectively.

As mentioned above, the CD16$^+$ NK cell subset is mainly involved in mediating ADCC. NK cell therapy with or without anti-gp120/41 antibodies may be more effective in restoring ADCC and controlling HIV replication in HIV-infected patients. Finally, several cytokines are known to increase ADCC against HIV-infected cells (reviewed in ref. [80]).

## HIV STRATEGIES TO EVADE NK CELL RESPONSES OF THE HOST

Viruses generally use multiple strategies to counter NK cell responses of the host. HIV is no exception. The strategies aimed at evading antiviral effects of the host's NK cell responses are listed in **Table 2** and are discussed below.

### Changing the expression of MHC and non-MHC ligands for NKRs

The down-regulation of MHC class I antigens on the surface of infected cells is a common strategy used by a variety of viruses

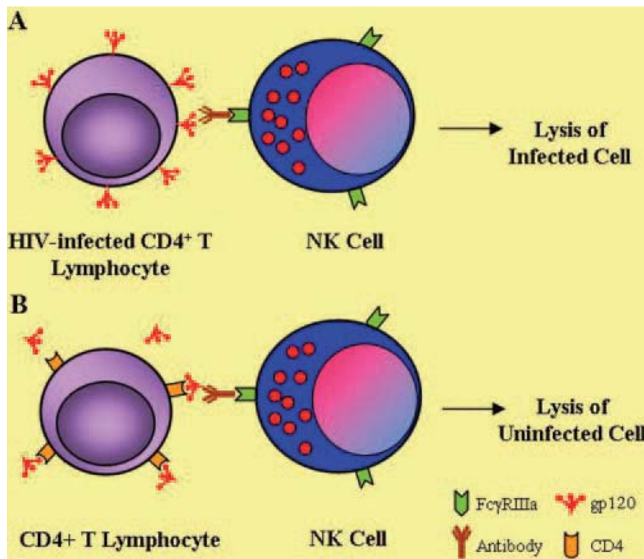


**Fig. 1.** Schematic representation of ADCC phenomenon. (A) NK cells kill HIV-infected CD4$^+$ T cells expressing gp120 via gp120-specific antibodies. The antibodies on one hand bind to gp120 and on the other hand, to CD16 on NK cells via their Fc regions. By killing HIV-infected cells, ADCC may help control the infection. (B) NK cells may also kill uninfected, gp120-bound CD4$^+$ T cells. The process may not discriminate between HIV-infected and gp120-bound, uninfected CD4$^+$ T cells.

to evade antiviral CTL responses of the host, as CTL recognize viral peptides in association with these antigens (reviewed in refs. [91–93]). A global decrease in the expression of MHC antigens, however, makes virus-infected cells susceptible to NK cell-mediated killing. Therefore, viruses have developed various strategies to evade this NK cell-mediated killing. Two HIV proteins have been shown to affect expression of MHC class I antigens: Tat represses promoters of the MHC class I and the β-2 microglobulin genes, and viral protein U (Vpu) interferes with an early step in the biosynthesis of MHC antigens [94, 95]. However, a global down-regulation of MHC class I antigens has rarely been observed in HIV-infected cells. Instead, several studies have documented that HIV differentially down-regulates the expression of MHC class I antigens on the surface of the infected cells. The viral protein Nef recognizes certain motifs present in the cytoplasmic tails of MHC class I antigens and causes their degradation. These motifs are present mostly in the cytoplasmic tails of HLA-A and -B but not of HLA-C and HLA-E antigens. Consequently, the expression of HLA-A and -B but not of HLA-C and -E is decreased on the surface of HIV-infected cells [96–98]. It is noteworthy that HLA-A and -B predominantly present viral peptides to CTL. Of these, only Bw4-serospecific HLA-A and HLA-B allotypes act as ligands for KIR3DL1. On the other hand, HLA-C and HLA-E present relatively fewer HIV-derived. immunodominant peptides to CTL. Nevertheless, all known HLA-C and HLA-E allotypes act as ligands for KIR and CD94/NKG2, respectively. From the perspective of NK cell functions, HLA-C and HLA-E are more important. The maintenance of these HLA molecules on the surface of HIV-in-

TABLE 2. How HIV Evades Host's NK Cell Response

**It does so by:**

1. Differential down-regulation of MHC class I antigens via the negative factor (Nef) protein.
2. Inducing and stabilizing the expression of HLA-E and -G in the infected cells.
3. Directly infecting and eliminating NK cells.
4. Blocking NK cell-mediated killing.
5. Promoting apoptosis of NK cells.
6. Dysregulating the expression of NKRs.
7. Dysregulating the production of NK cell-activating cytokine.
8. Down-regulating the expression of adaptor molecules involved in the signaling cascade of NK cell activation.
9. Modulating NK cell differentiation and maturation program.
10. Interfering with NK cell interactions with DC and other immunocytes.

fected cells protects them from NK cell-mediated lysis. A decreased expression of HLA-A and -B makes them invisible to most of the virus-specific CTL. However, it comes with some price. The infected cells become susceptible to killing by the NK cells expressing HLA-Bw4-specific KIR. By a differential modulation of HLA antigens on the surface of infected cells, virus evades most if not all CTL and NK cell-mediated killing. Indeed. autologous NK cells do not kill HIV-infected T cell blasts despite decreased HLA-A and -B antigens on their surface [99].

In addition to classical MHC class I antigens, HIV modulates expression of nonclassical MHC antigens. The infection increases the expression of HLA-E on the surface of $CD4^+$ T cells in in vitro experiments [55]. At least one potential mechanism of this increase is a peptide from the viral protein p24 (residues 14–22), which can bind and stabilize HLA-E on the surface of HIV-infected cells [100]. In line with these results, increased expression of HLA-E has been reported on the surface of $CD4^+$ T cells in HIV-infected persons. The increase was more pronounced in advanced stages of the infection and correlated with peaks in viremia [55].

Conflicting results have been reported concerning the effect of HIV infection on the expression of HLA-G. The infection was reported to cause down-regulation of HLA-G on the surface of HIV-infected cells in a Vpu-dependent manner [96, 101]. However, the molecule is expressed on monocytes and T lymphocytes in HIV-infected persons, probably as a result of HAART and increased concentrations of IL-10 in the circulation [102, 103]. HLA-G is normally expressed on certain immune-privileged sites, e.g., cornea, and on invading cytotrophoblasts in pregnancy and is believed to protect a developing fetus from the mother's immune responses (reviewed in ref. [104]). Increased expressions of HLA-E and -G on the surface of HIV-infected cells would increase their resistance to NK cell-mediated killing as well as to macrophage activation.

Although exact ligands for NCR are not known, Vieillard et al. [105] have reported increased expression of NKp44 binding to HIV-infected $CD4^+$ T cells. They have shown that a peptide (SWSNKS) derived from the transmembrane unit (gp41) of a viral envelope protein induces the unknown NKp44 ligand on $CD4^+$ T cells. It also suggests that the unknown ligand for this receptor could be a peptide-binding MHC or MHC-like molecule. The induction of NKp44 ligand on the HIV-infected cells may promote their killing by cytokine-activated NK cells. The increased expression of NKp44 ligands on the surface of HIV-infected cells has been corroborated by Ward et al. [83], who also reported increased expression of MHC class I chain-related protein A (MICA), MICB, and the human CMV (HCMV) glycoprotein UL16-binding protein 1 (ULBP-1), 2, and 3 on T cell blasts infected with HIV. They also reported a decrease in the expression of CD48 and NK-T-B antigen, and no change was observed in the expression of NKp30 and NKp46 ligands. As mentioned earlier, MICA, MICB, and ULBPs serve as ligands for NKG2D, which is an activating receptor expressed on all NK and $CD8^+$ T cells in humans. It is not in the interest of a virus to induce expression of these ligands on the surface of infected cells, as the virus-infected cells would be killed by NK cells as well as by $CD8^+$ T cells via NKG2D. Therefore, viruses have developed different strategies to evade this NKG2D-mediated killing. For example, HCMV encodes a glycoprotein UL-16, which can bind ULBPs intracellularly and prevent them from reaching cell surface and interacting with NKG2D [106]. Another protein from this virus, UL142, binds MICA and prevents its interaction with NKG2D [107]. Tumors may cleave and shed soluble MICA and MICB to interfere with NKG2D-mediated killing of tumor cells [108, 109]. HIV uses its Nef protein to evade NKG2D-mediated killing. The protein, in addition to down-regulating the expression of HLA-A, -B and CD1d, also binds to and degrades MICA, and ULBP-1 and -2 [110].

It is noteworthy that NKG2DL are usually induced in human cells upon genotoxic stress, which activates DNA damage response (reviewed in ref. [111]). The response arrests cell cycle until the damage is repaired. If the damage is not repairable, the response induces apoptosis in the cells. The response is initiated by two PI-3K-like kinases: ataxia telangiectasia mutated (ATM) and ATM and RAD-3-related (ATR). The two kinases are activated by dsDNA and ssDNA breaks, respectively. Stalled replication forks also activate ATR. The induction of NKG2DL by HIV implies that the infection causes genotoxic stress in the infected cells. It could be an unintended consequence of the functional activities of the viral protein R (Vpr). The protein is known to induce cell-cycle arrest by recruiting DCAF-1/VprBP and an E3 ligase Cul4-DDB1 in eukaryotic cells and activates ATM and ATR, which may result in the induction of NKG2DL [112]. **Figure 2** summarizes HIV-induced changes in the expression of MHC ligands in HIV-infected persons.

## Changing the expression of NKRs

Viruses may evade NK cell-mediated killing by increasing the expression of inhibitory and/or by decreasing the expression of activating receptors on the surface of NK cells of the infected host. There is sufficient evidence to suggest that HIV uses this strategy to counter antiviral NK cell responses of the host. Several workers have documented an increase in the expression of inhibitory receptors (e.g., iKIR) and a decrease in the expression of activating receptors (e.g., NCR) in HIV-infected individuals. Interestingly, these dichotomous effects on inhibitory and activating NKRs were mainly observed in viremic patients and correlated with viral load. Only a transient decrease was observed in the expression of 2B4, whereas no effect was observed on the expression of NKG2D on NK cells from HIV-infected persons. These changes in receptor expression were often accompanied with decreased cytolytic activities of NK cells [1, 16, 49, 51] (reviewed in ref. [113]). The occurrence of these changes in viremic patients as well as their correlation with viremia suggest that the virus might have caused the receptor modulations. This is further supported by the fact that a stabilizing effect of the HAART treatment on the receptor expression was observed. However, the treatment was able to restore the expression to normalcy after a long period of administration, when it resulted in undetectable viral loads in the patients [51, 56]. In addition to direct effects of the virus, chronic activation of the immune system via antigens from HIV-1 and/or from other coinfecting pathogens may have

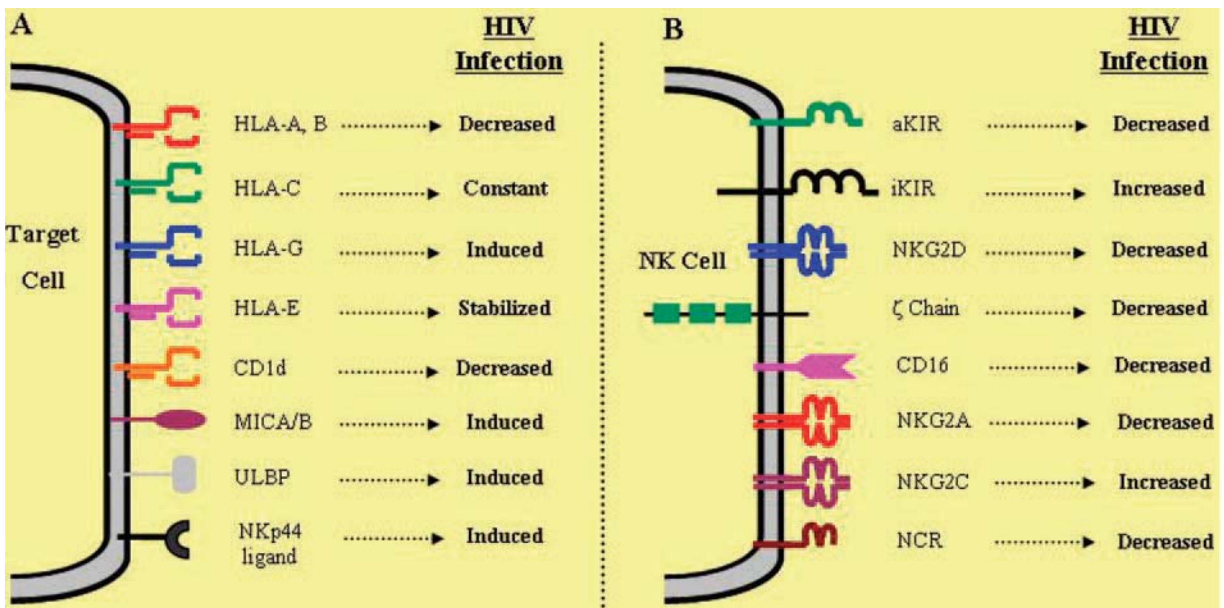


**Fig. 2.** HIV-induced changes in the expression of NKRs and their ligands. (A) Changes in the expression of various NKR ligands. (B) Changes in the expression of NKRs from HIV-infected persons. Collectively, these changes may protect HIV-infected cells from NK cell-mediated lysis. aKIR, Activating KIR; iKIR, inhibitory KIR.

caused perturbations in the expression of NKR. Repeated antigenic stimulations are known to induce expression of several inhibitory receptors including KIR on immune cells [114].

With respect to the NKG2/CD94 family receptors, an expansion of CD94/NKG2C$^+$ and a marked depletion of CD94/NKG2A$^+$ NK cells have been described in the peripheral blood of HIV-infected persons [53]. HAART did not reverse these changes despite reducing viremia to undetectable levels in these patients. These changes are also not observed in the individuals infected with HIV alone. It is noteworthy that similar changes in the expression of the CD94/NKG2 family of receptors have been reported in humans suffering from chronic infections with HCMV. It seems more likely that HCMV infection may be the real cause in driving these changes in HIV- and HCMV-coinfected patients. Indeed, HCMV-infected fibroblasts cause proliferation of NKG2C$^+$ human NK (HNK) cells in in vitro studies. However, the changes seem to be more pronounced in the coinfected individuals [53, 115, 116]. These observations suggest a possible role of HIV infection in these NKR perturbations. It may be relevant to mention here that NKG2A is an inhibitory and NKG2C is an activating NKR. Both of them bind to HLA-E on target cells and regulate NK cell functions. The two receptors usually occur on the CD56$^{high}$CD16$^{low}$ subset of NK cells, which express low levels of KIR. It is believed that NKG2A may be important in maintaining self-tolerance in NK cells that do not express self-reactive KIR. It is noteworthy that a HCMV-encoded protein UL40 provides a peptide, which binds and stabilizes HLA-E. The HLA-E is also stabilized by a peptide derived from the HIV p24 protein [100, 117]. An increased expression of HLA-E on CD4$^+$ T cells in HIV-infected individuals has also been described [55]. The enhanced HLA-E expression may have caused proliferation of NKG2C$^+$ and/or an early depletion of NKG2A$^+$ NK cells. It is not clear how these receptor changes could affect progression of HIV infection. The fact that persons coinfected with HIV and HCMV progress more rapidly toward AIDS [118] suggests that NKG2C$^+$ NK cells may be involved in immunopathology. We speculate that these CD56$^+$NKG2C$^+$ NK cells may kill many different types of cells including mature DC and CD4$^+$ T cells, which express HLA-E [100, 117, 119]. However, it must be emphasized that there is no direct experimental evidence at this point in time to support this notion. The modulation of NKRs by HCMV provides an example of how this herpesvirus may affect the natural course of HIV infection in coinfected individuals.

NKp44 is an activating receptor, which is not expressed on resting NK cells. The receptor is induced on cytokine-activated NK cells. A group of researchers has shown that freshly isolated NK cells from HIV-infected viremic persons are aberrantly activated: They are CD69$^+$, HLA-DR$^+$ but do not express NKp44. Furthermore, they express relatively low levels of other NCR [52].

As mentioned above, HIV or its products have been implicated in the induction of changes in the expression of NKRs in HIV-infected patients. It is noteworthy that the infection causes a dysregulated production of many cytokines in the human body. It is not surprising that these cytokines have been implicated in this process. Two groups of researchers have suggested the involvement of IL-10. This immunosuppressive cytokine induces changes in the expression of NKRs in vitro similar to those seen in HIV-infected patients in vivo, i.e., increased expression of CD94, CD161, and CD158a or KIR2DL1 [41, 48]. It is noteworthy that concentrations of IL-10 are increased in the circulation of HIV-infected persons. The changes observed in the expression of NKRs in HIV-infected persons are summarized in Figure 2.

## Changing the expression of NKRs on non-NK cells

Many NKRs are also expressed on non-NK cells. CD56 is usually expressed on activated $CD8^+$ T lymphocytes. Its expression has been associated with the acquisition of cytotoxic functions in these cells [120]. A decrease in the expression of CD56 has been described on NK and $CD8^+$ T cells in HIV-infected persons. Indeed, $CD56^+$ NK and $CD8^+$ T cell populations from HIV-infected persons express less perforin and are less cytolytic compared with their counterparts from HIV-seronegative, healthy subjects [10, 58, 121].

Normally, monocytes do not express CD16; they do so upon activation. TGF-β1 has been shown to induce its expression on monocytes in humans. Monocytes from HIV-infected AIDS patients express this marker, and this expression correlates with increased concentrations of this cytokine in the circulation of these patients [122]. This expression has implications for virus replication, as $CD16^+$ monocytes are highly permissive to HIV replication [123]. Furthermore, these cells may shed sCD16, which may interfere with killing HIV-infected cells via ADCC.

CD57 (Leu-7; HNK-1) is a 110-kD glycoprotein expressed on a subset of NK, $CD8^+$, and $CD4^+$ T cells, which plays a role in homotypic cell adhesion. It bears a sulfated carbohydrate epitope (glycotope), which is also present on several other glycoproteins and glycolipids expressed on the surface of different cell types. The epitope is regulated by two glucuronyltransferases (-P and -S) and a sulfotransferase (HNK-1; see ref. [124] for a review). In the immune system, CD57 is expressed on terminally differentiated effector cells. These cells can neither proliferate nor circulate; however, they do migrate to nonlymphoid tissues and secrete cytokines. In the case of $CD8^+$ T cells, CD57 expression is restricted to effector/memory phenotype. The marker is also expressed on aberrantly differentiated and clonally exhausted effector cells. Increased numbers of CD57-expressing NK and $CD8^+$ T cells occur in chronic viral infections including that of HIV [16, 124]. Repeated antigenic stimulation may lead to clonal exhaustion and increased CD57 expression in HIV-infected persons. Furthermore, aberrant differentiation of these cells as a result of a lack of CD4 help and/or dysregulated production of cytokines such as IL-2, IL-7, IL-15, IL-21, IL-10, etc., may also lead to an increased number of $CD57^+$ cells in this infection. Increased numbers of $CD57^+$ T and NK cells represent immune dysfunction.

In the course of normal differentiation into terminally differentiated effector cells, $CD8^+$ T cells acquire CD57 and lose CD27. However, in the case of HIV infection, they acquire relatively low levels of CD57 (compared with HCMV and EBV-specific effector CTL) and do not lose CD27 expression [125]. This suggests that HIV-specific CTL undergo aberrant and incomplete course of differentiation. This defective differentiation of HIV-specific CTL is further supported by their decreased expression of perforin, lower cytotoxicity, and increased expression of the inhibitory marker programmed death (PD)-1 and other phenotypic markers [126, 127]. It has also been proposed that in HIV infection, NK cells and CTL undergo premature senescence without undergoing complete physiological differentiation. This premature senescence has been proposed as the main reason of inability of HIV-infected persons to control the virus [128].

A subset of CTL has been shown to express KIR, NKG2/CD94, killer lectin-like receptor (KLR)-G1, and ILT-2. These markers are usually expressed at distinct stages in the course of development and differentiation of naïve CTL into effector/memory cells. For example, KLR-$G1^+$ $CD57^+$ CTL represent terminally differentiated effector CTL, and $KLRG1^+CD57^-$ CTL represent long-lived memory CTL [129]. Developing T cells acquire these markers after completion of their TCR gene rearrangements. Therefore, CTL with similar TCRVβ genes may have different repertoires of KIR, NKG2, and KLR-G1 receptors [130]. The level of expression of these receptors on CTL determines their antigenic threshold for activation and is "fine-tuned" to avoid autoimmunity and to mount an effective immune response against invading pathogens [131, 132]. The expression of KIR in humans (and of Ly49 in mice) seems to confer survival advantage in CTL and prevents them from undergoing activation-induced cell death in response to TCR stimulation. $KIR^+$ CTL express higher levels of the antiapoptotic protein Bcl-2 as compared with the ILT-$2^+$ CTL [133–136]. It appears that KIRs are expressed on long-lived memory T cells having monoclonal or oligoclonal expression of TCRVβ genes. ILT-2, on the other hand, are expressed earlier than KIR in the course of differentiation of CTL. Consequently, they are expressed on a larger percentage of antigen-specific CTL with a broader use of TCRVβ genes. Interestingly, ILT-$2^+$ but not $KIR^+$, HIV-specific CTL could be easily detected in HIV-infected AIDS patients, which again suggests their defective differentiation. $KIR^+$ CTL express perforin and secrete IFN-γ, whereas ILT-$2^+$ CTL can only secrete cytokines and contain little perforin [133]. Expansions of $CD8^+$T cells expressing these receptors usually occur in viral infections, which subside upon resolution of the infection. However, increased frequencies of the cells bearing these receptors persist in chronic infections [137–140]. The expression of inhibitory NKRs on CTL may be essential for the development of virus-specific memory responses. This expression raises the activation threshold of CTL and prevents indiscriminate killing of host cells but still allows killing of virus-infected cells. However, coengagement of inhibitory receptors inhibits TCR-mediated activation of CTL [141]. It has also been observed in in vitro studies that a blockage of KIR markedly increases CTL-mediated killing of HIV-infected, autologous cells [40]. In mice, which do not have KIR genes but express their functional orthologs (LY49 genes) on their NK cells and a subset of CTL, it was also demonstrated that blockage of LY49 receptors increases anti-tumor activities of NK cells resulting in tumor regression [142, 143]. Interestingly iKIR, CD94/NKG2, and KLR-G1 could also be detected but sparsely on $CD4^+$ T cells in human peripheral blood. Percentage of these cells increases with age.

It is noteworthy that many of the observations concerning the expression of inhibitory receptors on $CD8^+$ T cells have been verified in vivo in mice infected with chronic lymphochoriomenengitis virus infection [144].

It has been well documented that HIV infection induces a vigorous antiviral CTL response in the host (reviewed in ref.

[145]). The frequency of virus-specific CTL in the circulation of HIV-infected persons is usually higher as compared with that seen in several other viral infections. Consequently, HIV-specific CTL can be readily demonstrated in the peripheral blood of HIV-infected individuals without prior stimulation and expansion. Despite this, cellular immune response is unable to control HIV infection in humans. There could be several reasons for the inability of the antiviral CTL responses to clear HIV infections: high mutability of HIV-1, depletion of $CD4^+$ T cells and consequent loss of CD4 help, incomplete differentiation of CTL, increased expression of proapoptotic molecule PD-1, impaired proliferative capacity of HIV-specific CTL, decreased expression of CD3 on CTL, etc. (reviewed in refs. [145–147]). An increased expression of inhibitory NKR on these cells may also play a role in the ultimate failure of this antiviral immune response in controlling HIV infection in humans. This is supported by the facts that long-term, nonprogressors do not express increased levels of these receptors on their $CD3^+CD8^+$ peripheral blood cells, and in vitro blocking of these receptors causes increased killing of the CTL against autologous, HIV-infected cells [45, 148].

## Disturbing NK cell interactions with other immunocytes

As mentioned earlier, NK cells interact intimately with DC. These interactions have important implications for the ensuing innate and adaptive immune responses against viral infections and malignancy. During these interactions, the two types of cells form an immune synapse with each other. NK cells induce polarized secretion of IL-12, IL-18, and membrane-bound IL-15 from DC. The polarized secretion from DC requires tubulin rearrangement and activation of calcium-calmodulin-dependent kinase II (CAMK-II) [149, 150]. These cytokines activate NK cells, which in return, secrete IFN-γ, TNF-α, and high mobility group box-1 (HMGB1), which cause DC maturation [151, 152] (reviewed in ref. [153]). It is noteworthy that HMGB1 is the most potent proinflammatory cytokine that causes DC maturation. The DC-maturing capacity of different NK cell clones depends on their ability to secrete this cytokine [154, 155]. The physical contact between the two cell types involves interactions among several receptor-ligand pairs, which include LFA-1, NKp30, NKp46, 2B4, DNAX accessory molecule 1 (DNAM-1), NKG2D, TNFRII, and NKG2A [150, 156, 157]. NK cells also perform the task of quality control and kill immature DC if they do not undergo proper maturation. It has been demonstrated that NKp30, DNAM-1, and LFA-1 are involved in the NK cell-mediated killing of autologous, immature DC [158]. Mature DC are not killed, as the maturation process induces expression of HLA antigens, which protect them from NK cells. It is noteworthy that it is the $CD56^{high}CD16^{dim}$ NK cells that interact with and cause maturation or killing of immature DC. These NK cells express little KIR and express CD94/NKG2A as the main inhibitory receptors. It is not yet fully understood how NK cells choose between killing and causing maturation of immature DC. It probably depends on the profile of expression of several molecules on the surface of immature DC. If DC fail to express HLA antigens upon maturation, they may be killed by NK cells. The NK cell-activating potential of DC also depends on the milieu in which they differentiate. For example, immature DC, differentiated in the presence of IL-4, selectively activate NK cells but not T cells. IL-4 induces the expression of triggering receptor expressed on macrophage-2 on DC [159, 160]. The ratio between NK cells and their interacting DC is also a factor: A greater ratio tends to favor the killing rather than maturation.

After maturation, DCs express CCR7 and migrate to secondary lymph organs, e.g., lymph nodes, where they interact with T cells as well as with activated NK cells, which control and determine T cell-priming capabilities of DC. The DC generated from monocytes in the absence of NK cells are unable to prime $CD8^+$ T cells. The NK/DC interactions may allow DC to prime T cells without help from $CD4^+$ T cells. The speculation is that NK cells may themselves provide this help. As mentioned earlier, properly activated NK cells express molecules that may enable them to interact with T cells. The cross-talk also involved cell–cell contact via CD161/Clr-b, 2B4/CD48, DNAM-1/Poliovirus receptor, NKG2D/NKG2DL, as well as soluble mediators, e.g., TNF-α, IFN-γ, IL-12, and others [161–163].

Depending on these interactions, DC may emerge that could prime naïve $CD4^+$ T cells into TH1-type cells. The interactions may also lead to the generation of DC, which may favor the generation of immunosupressive regulatory T cells (Tregs).

As a result of the importance of NK/DC interactions in mediating effective antiviral immunity, viruses may target these interactions for immune evasion. For example, it has been shown that monocyte-derived DC (MDDC) from hepatitis C virus (HCV)-infected persons and their autologous NK cells fail to induce reciprocal activation. This failure results from the inability of these MDDC to express MICA and MICB in response to IFN-α. The MDDC generated from the infected persons produce more IL-10 and TGF-β [164, 165], and TGF-β promotes induction of IL-10-secreting Tregs by inducing forkhead box P3 (FoxP3) expression in $CD4^+$ precursor cells [166].

The NK/DC interactions also become aberrant in HIV-infected persons. The NK cell-editing function seems to be lost in HIV-infected persons. Activated NK cells from viremic persons are unable to kill autologous, immature MDDC [51, 167, 168]. This defect was more profound in the $CD56^-CD16^+$ NK cell subset, as it could not be overcome even after masking NK cell inhibitory receptors. The mature DC from HIV-infected persons produced less IL-12 and could not activate interacting NK cells. Consequently, these NK cells produce less IFN-γ. Defective NKp30- and TRAIL-mediated killing was blamed on the escape of the immature DC from NK cell-mediated killing in HIV-infected persons [167]. Aberrant NK cell/DC interactions may result from overall defective NK cell functions, depletion of certain functional NK cell subsets, and changes in the expression of NKRs and coreceptors. Certain viral proteins have also been shown to interfere in these interactions. It was demonstrated in in vitro studies that LFA-1-mediated activation of CAMK-II and microtubule rearrangement are essential for NK cell activation by mature DC. Tat inhibits this activation by interfering with $Ca^{++}$ influxes and activation of CAMK-II. More specifically, the C-terminal domain of Tat was found to be responsible for this interference [150]. In another study, Nef was shown to dysregulate DC/NK

interactions. Nef-pulsed DC inhibit chemokine secretory capacity as well as the cytotoxic ability of NK cells, including the $CD56^{low}$ $CD16^{high}$ subset, possibly by inducing TGF-β and IL-10 [169].

NK cell/DC interactions determine T cell-priming characteristics of DC. For example, IFN-α-activated NK cells induce type 1 DC. These DC, which produce IL-12 upon stimulation via CD40, are efficient in priming TH1-type $CD4^+$ effector T cells. IFN-α is necessary for inducing this kind of helper function in NK cells [170]. Treatment of NK cells with IL-2 or polyinosinic:polycytidylic acid has similar effects [152, 171]. Improperly "helped" DC may induce tolerance in the interacting T cells and/or may cause their differentiation into suppressive Tregs. The DC, which fail to prime T cells, frequently express tryptophan-catabolizing enzyme indoleamine 2,3-dioxygenase (IDO), which converts tryptophan (an essential amino acid) into kynurenine and other catabolites [172, 173]. In the absence of tryptophan, T cells cannot proliferate. Furthermore, tryptophan catabolites induce apoptosis in T cells [174]. This results in decreased serum concentrations of tryptophan. It has also been shown that individuals with increased IDO activity are compromised in the production of 5-hydroxytryptamine in their brains. This mediator is important for signaling across neuron synapses. Its decreased production leads to decreased cognitive abilities, loss of memory, depression, and other psychiatric abnormalities. In the case of HCV-infected persons, it has been shown that decreased serum concentrations of tryptophan correlate with psychiatric symptoms in these patients. Decreased serum concentrations of tryptophan have also been reported in HIV-infected persons [175]. These concentrations correlate with neoptrin as well as with depression, psychiatric, and neurological symptoms. Abnormal NK/DC interaction could play a role in these manifestations.

As mentioned above, NK cells also interact with macrophages. NK cell/macrophages could play an important role in protecting host from pathogens (reviewed in ref. [176]). Nothing is known about NK cell/monocyte interactions in HIV infections.

Tregs are $CD4^+CD25^+FoxP3^+$ and glucocorticoid-induced TNFR-related $protein^+$ T cells known for their immunosuppressive properties. They can inhibit immune responses by suppressing T and NK cell functions. Enhanced numbers and functional activities of Tregs have been reported in the tissues of HIV-infected persons (reviewed in ref. [177]). It has been demonstrated that Tregs suppress NK cell functions. Activated Tregs express membrane-bound, functionally active TGF-β. In vitro incubation of Tregs with NK cells leads to down-regulated expression, which is TGF-β-dependent, of NKG2D and other activating receptors on NK cells (reviewed in ref. [178]). Depletion of Tregs may represent a novel way for enhancing NK cell functions in HIV-infected persons. It also leads to enhanced, HIV-specific CTL activity [177].

## Dysregulating production of NK cell-activating cytokines in HIV-infected individuals

NK cells bear receptors for a variety of cytokines, e.g., IL-2, IL-12, IL-15, IL-18, IL-21, TGF-β, type I IFNs (IFN-α/β), etc. An optimum production of these cytokines is needed to maintain NK cell homeostasis and ready-to-kill state in the body. Several studies have shown that hosts (including humans) respond to a viral infection with the enhanced production of several cytokines, e.g., IFN α/β, IL-12, IL-15, and IL-18 [179, 180]. A coordinated production of these cytokines is essential for mediating an effective antiviral NK cell response of the host. Immediate activation of NK cells following a viral infection is, to a large extent, a consequence of this virus-mediated cytokine production. Each of these cytokines plays a distinct role in NK cell activation and expansion following a viral infection. In chronic viral infections, e.g., HIV-1, this coordinated production of cytokines is dysregulated, which may be responsible, at least in part, for defective NK cell responses. **Table 3** shows how these cytokines affect NK cell function and what happens to their production in this viral infection. HIV and its proteins play a role in the dysregulated production of cytokines. For example, gp120 induces IL-10, IFN-β, and TNF-α, and Tat induces TGF-β1 and IL-6 but inhibits IL-12 production in human PBMC [181–183]. The viral protein Nef induces IL-15 and decreases IL-18 production in the human cells [184, 185]. Overall, it has been well-documented that HIV-infected persons become compromised in their ability to produce IL-2, IL-12, IL-15, and IL-21 [179, 186–189]. Their

TABLE 3. Effects of Different Cytokines on Human NK Cells

| Cytokine | Development Maturation | Cytotoxicity | Survival Homeostasis | Cytokine Production | Proliferation | Status in HIV[a] |
|---|---|---|---|---|---|---|
| IFN-α/β | – | ↑ | –/+ | ↑ | –/+ | ↓ |
| IL-1 | – | ↑ | –/+ | ↑ | –/+ | ↓ |
| IL-2 | – | ↑ | –/+ | ↑ | ↑ | ↓ |
| IL-7 | – | ↑ | + | ↑ | ↑ | ↓ |
| IL-4 | D2 | –/+ | – | –/+ | ↑ | ↑ |
| IL-10 | – | ↑ | – | ↑ | –/+ | ↑ |
| IL-12 | D1 | ↑ | ↑ | ↑ | ↑ | ↓ |
| IL-15 | + | ↑ | ↑ | ↑ | ↑ | ↓ |
| IL-18 | M | F | ↑ | ↑ | ↑ | ↑ |
| IL-21 | M | ↑, N | ↑ | ↑ | ↑ | ↓ |
| TGF-β | – | ↓ | ↓ | ↓ | ↓ | ↑ |

It is noteworthy that cytokines usually act in synergism with each other; e.g., IL-2 and IL-12 act synergistically with IL-12 and IL-18, respectively. ↑, Increased effect, ↓, decreased effect; –, no effect; –/+, the cytokine may have an enhancing effect in combination with another cytokine; D1, differentiation into NK1; D2, differentiation into NK2; F, increased cytotoxicity as a result of increased expression of Fas ligand (FasL); N, decreased expression of NKG2D and decreased cytotoxicity against NKG2DL-positive target cells; M, cytokine needed for full maturation of NK cells. [a]Overall production in HIV-infected persons.

type I IFN-producing cells (pDC) also produce less of the cytokine and are progressively depleted [190, 191]. A lack of these cytokines affects differentiation, survival, and cytolytic functions of NK cells. On the other hand, the concentrations of some immunosuppressive cytokines, e.g., TGF-β and IL-10, are increased in the circulation of HIV-infected patients [192, 193]. Parato et al. [48] have proposed that increased IL-10 induces similar changes in NK cells as observed in HIV-infected persons. They observed a normalizing effect of HAART on IL-10 and NK cell phenotypes in a limited number of HIV-infected persons. Contrary to IL-12 and IL-15, whose production decreases in HIV-infected persons, we and others [194, 195] have reported increased concentrations of IL-18 in the sera from HIV-infected persons. Interestingly, the PBMC from these persons were found to produce less of this cytokine with or without stimulation with LPS. Interestingly, cells other than monocytes also produce the cytokine: Keratinocytes, adrenal cortex, and platelets also are rich sources of this cytokine. We have shown that activated platelets contribute toward increased concentrations of this cytokine in HIV-infected persons [196]. It is noteworthy that IL-18 concentrations also increase in chronic inflammatory conditions. The cytokine increases FasL expression on NK cells, and FasL-positive NK cells may be involved in fratricidal killing of other NK cells. The cytokine appears to hasten NK cell death via Fas/FasL interactions. In constrast to the concentrations of various cytokines, little is known whether there is any change in the expression of cytokine receptors on NK cells in HIV-infected patients. In this regard, a group has shown decreased expression of the IL-7R on NK cells in HIV-infected persons [197]. IL-7 promotes proliferation of the $CD56^{bright}$ subset of NK cells, which express this receptor. The viral protein Tat is known to down-regulate this receptor in $CD8^+$ T cells [198] and is probably also responsible for this effect in NK cells. The protein is released from HIV-infected cells and is actively taken up by other cells in the body. Another research group has documented that NK cells from HIV-infected persons do not respond to IFN-α [43]. The authors did not find out whether the lack of response was a result of a decrease in the cytokine receptor and/or a result of a defective signaling pathway of the cytokine. The decreased expression of cytokine receptors may adversely affect NK cell functions in a variety of ways: causing aberrant expression of NKRs, inducing apoptosis, etc.

## Altering NK cell-secreted cytokines and chemokines in HIV infection

The profile of NK cell-secreted cytokines appears to be modified in HIV-infected persons. As stated above, NK cells are known to secrete several cytokines and soluble mediators: IFN-γ, TNF-α, TNF-β, GM-CSF, IL-3, IL-4, IL-5, TGF-β1, IL-10, IL-13, etc. They do so upon interaction with the target cells, which trigger NK cell cytotoxicity as well as upon activation with an appropriate combination of other cytokines, e.g., IL-12 and IL-15. Interestingly, IL-15 appears to be required by NK cells for their production of TH2-type cytokines [199–203]. NK cells also express constitutive, but not inducible, endothelial NO synthase (NOS) and secrete NO. Interestingly, NOS inhibitors can significantly inhibit functions of HNK cells [204]. Several studies have shown that the profile of NK cell-secreted cytokines depends on the milieu in which they develop and differentiate. In analogy to TH1- and TH2-type $CD4^+$ T cells, NK cells could differentiate into type 1 or type 2 NK cells (NK1 or NK2). NK1 cells predominantly secrete IFN-γ, whereas NK2 cells predominantly secrete IL-5 and IL-13 [205, 206]. It is noteworthy that existence of the two types of NK cells has been demonstrated in vivo in humans, and they may affect the course of certain disease conditions. For example, NK1 and NK2 cells have been associated with episodes of relapses and remissions in multiple sclerosis, respectively [207]. It has also been shown that NK2 cells play a role in the immunopathogenesis of asthma and in the maintenance of normal pregnancy in humans [208, 209].

Surprisingly, we could not come across any study in literature about the profile of NK cell-secreted cytokines in HIV-infected persons. However, Chan et al. [210] have shown that NK cells from these persons are of type 2. Their study relied on two cell surface markers belonging to the IL-1R superfamily, IL-18R and ST2L, which are expressed on the surface of cells producing TH1- and TH2-type cytokines, respectively [210, 211]. These results support earlier reports implicating TH2-type cytokine responses in the immunopathogenesis of AIDS [193, 212, 213]. NK cells may be contributing to the predominance of TH2 cytokine responses in HIV-infected AIDS patients. However, studies are needed to investigate NK cell-secreted cytokines in humans in the course of HIV infection.

In addition to cytokines, NK cells produce abundant amounts of several chemokines, e.g., CCL3 (MIP-1α), CCL4 (MIP-1β), and CCL5 (RANTES), which play an important role in initiating NK cell-mediated inflammation. These chemokines are also important in the context of HIV infection, as they bind to CCR5 and block entry of CCR5-using M-tropic HIV strains from entering into human cells. It is important to note that primary HIV infections usually result from M-tropic viral strains. This may also explain why persons with high-activity NK cells may be relatively protected from contracting HIV infections [12, 13]. It has been demonstrated that NK cells from HIV-infected individuals produce relatively less amounts of these chemokines and may be less efficient in blocking CCR5 and suppressing HIV replication [6, 8]. Not surprisingly, culture supernatants of NK cells from HIV-infected persons are less efficient in suppressing HIV replication than similar supernatants obtained from the cells of HIV-seronegative, healthy persons. Interestingly, viremia seems to directly suppress chemokine production from NK cells [11].

## Infecting NK cells

Infecting the very immune cells that may inhibit viral replication is a clever strategy to evade host immunity. By infecting an immunocyte, the virus could cripple its immune effector functions. HIV-1 can infect many types of immune cells, e.g., $CD4^+$ T cells, macrophages, DC, etc. In vitro studies have shown that the virus can also infect NK cells [214, 215]. The $CD8^+$ NK cell subset was found to be more susceptible to HIV infection than the $CD8^-$ subset. The two cell subsets varied in the production of cytokines: the former producing more TNF-α and the latter producing more IFN-γ. This differential production of the cytokines was shown to be responsible for the differential susceptibility of the NK cell subsets to HIV infec-

tion [214, 215]. This preferential infection of $CD8^+$ NK cells with HIV-1 may also explain why the $CD8^+CD16^+$ NK cells are frequently depleted in the circulation of HIV-infected individuals [14, 27, 216]. The infected NK cells become impaired in their cytolytic functions. Remarkably, NK cells can also be infected with HIV with help from human herpesvirus 6 (HHV-6). The latter virus infects human NK cells and induces the expression of CD4 in these cells, rendering them susceptible to infection with HIV-1 [217]. It is noteworthy that HIV-infected individuals suffer from frequent reactivations of herpes viruses, and HHV-6 infection is considered an important cofactor in the development of AIDS. Moreover, CD8-tropic HIV-1 strains have also been isolated from HIV-infected AIDS patients. Interestingly, these strains use CD8 and not CD4 as a primary receptor in human cells and infect $CD8^+$ T cells [218]. Little is known about these CD8-tropic HIV strains. Theoretically, they could potentially infect $CD8^+$ NK cells. It is noteworthy that in vivo infection of NK cells in HIV-infected persons has also been demonstrated [219]. A small percentage (0.3–6.5%) of circulating $CD3^-CD56^+$ HNK cells expresses CD4 and HIV coreceptors, CXCR4 and CCR5. Proliferative activation of NK cells causes an increase in the expression of CD4 and CCR5 on these cells. $CD4^+$ NK cells can be infected in vitro with T- and M-tropic HIV-1 strains. A more efficient way of infecting NK cells is their coculture with HIV-infected cells. This suggests that in vivo, cell-to-cell infection of NK cells may be more important. NK cells seem to be relatively resistant to killing by HIV infection. The infected NK cells may persist in vivo despite treatment of the infected persons for several years with HAART [219–221]. Thus, NK cells may provide a sanctuary to HIV, and the virus-infected NK cells may represent important viral reservoirs. The virus may persist in these cells even when HAART may have reduced viremia to very low or undetectable levels. It may be relevant to mention here that NK cells express a higher level of P-glycoprotein compared with other lymphocytes. Therefore, HIV-infected NK cells may be relatively more resistant to antiretroviral drugs, e.g., protease and RT inhibitors [219, 222]. These findings have implications for therapeutic strategies being used for elimination of the virus from HIV-infected persons.

## Enhancing apoptosis in NK cells

NK cells from HIV-infected individuals have a reduced capacity to proliferate upon in vitro culture. It is noteworthy that the expression of a senescence marker CD57 is significantly increased on the surface of NK cells in HIV-infected persons. NK cells also undergo enhanced, spontaneous apoptosis as compared with the cells from healthy, control subjects. The enhanced apoptosis was ascribed to their relatively low expression of the antiapoptotic proteins Bcl-2 and Bcl-$X_L$. It has been shown that Tat induces TGF-β and apoptosis in NK cells. It also down-regulates Bcl-2 expression in other hematopoietic cells [183, 223]. IL-10 is known to enhance serum starvation-induced apoptosis in human cells by decreasing transcription of antiapoptotic proteins Bcl-2 and Bcl-$X_L$. As mentioned above, increased concentrations of this cytokine in the circulation of HIV-infected persons have been well documented. The viral glycoprotein gp120 from T-tropic viral strains has also been shown to increase expression of proapoptotic genes and decrease expression of antiapoptotic genes in NK cells [224]. In this connection, another study has shown that gp120 interaction with the viral coreceptor CXCR4 induces cell death via autophagy: a kind of programmed cell death in which large chunks of cellular material and cytoplasmic organelles are degraded in lysosomes [225]. NK cells constitutively express this receptor, and its interaction with gp120, which is present in virions and/or the circulation, may induce autophagic death of NK cells. As mentioned above, recombinant gp120 has, in fact, been shown to induce up-regulation of several proapoptotic genes in NK cells [224]. So, it is not surprising that viremia is associated with decreased NK cell numbers as well as with decreased functional capability of NK cells in HIV-infected persons [1].

Addition of the prosurvival cytokine IL-15 to in vitro NK cell (and T) cell cultures increases their survival by up-regulating the expression of Bcl-$X_L$ [226]. In normal NK cells, which constitutively express high amounts of Bcl-2 and Bcl-2-like proteins, IL-15 increases NK survival by down-regulating Bim and maintaining antiapoptotic protein Mcl-1 [227]. Bim is the only-BH-3 domain-containing, proapoptotic member of the Bcl-2 family of proteins. It binds with and inactivates Mcl-1, another member of the Bcl-2 family having antiapoptotic functions (reviewed in ref. [228]). Recombinant human IL-15 may represent a useful immunotherapeutic tool and vaccine adjuvant for HIV-infected AIDS patients because of its prosurvival and antiapoptotic effects on NK cells, less toxicity, and minimal enhancement of HIV replication (reviewed in ref. [229]).

A small proportion of NK cells from normal, healthy persons undergoes apoptosis when they are used as effector cells in in vitro NK cell cytotoxicity or ADCC assays [230, 231]. It has also been demonstrated that NK cells can undergo apoptosis after activation, as in the case of T cells. For example, IL-2 and IL-12-stimulated NK cells undergo apoptosis when they were incubated with immobilized antibodies directed against CD16, CD2, or CD94 [232–234]. It was also learned that incubation of NK cells with high concentrations of certain activating cytokines, e.g., IL-15 and IL-12, induced production of TNF-α, which caused apoptosis of NK cells [235]. In fact, it is a negative-feedback mechanism by which NK cells control and limit self-activation and secretion of IFN-γ. As mentioned above, we and others [194, 195] have reported increased concentrations of IL-18 in the sera from HIV-infected persons. The cytokine induces FasL expression on NK cells, which could lead to fratricidal killing of NK cells via Fas/FasL interactions. This may explain a negative correlation between serum concentrations of IL-18 and NK cell numbers reported in patients suffering from chronic autoimmune disorders [236]. Indeed, we have also observed a significant negative correlation between serum IL-18 concentrations and NK cell numbers in these individuals (unpublished data). These studies suggest that IL-18 may be associated with compromised NK cell functions in HIV infections. The HIV protein Tat, secreted from HIV-infected cells, has been shown to induce FasL expression on NK cells and CTL [237]. It is tempting to speculate that Tat and IL-18 may act in concert to induce FasL expression on NK and CTL in HIV-infected persons. Fas/FasL interactions have

been implicated in the immunopathogenesis of AIDS in HIV infection (reviewed in ref. [238]).

## NK CELL FUNCTIONS BECOME COMPROMISED IN HIV-INFECTED PERSONS

Although humans respond to HIV infection with activation of their NK cells, the virus uses many different strategies to neutralize this host response. As a consequence, NK cell function becomes compromised in these infections. Several workers have reported that NK cell functions (killing of target cells, ADCC effector function, editing of DC, and production of cytokines and chemokines) become defective in HIV-infected persons [26, 33, 34, 37, 38, 63, 239, 240]. The defects in the NK cell compartment usually occur in early stages of the infection. A decreased expression of LFA-1 on cells from HIV-infected persons has been reported [241]. Furthermore, it has also been shown that immune activation leads to an increase in shedding of soluble ICAMs and CD16 in the circulation of HIV-infected persons. The soluble forms of these molecules interfere with their membrane-inserted forms. The role of LFA-1 and its ligands in cell adhesion, conjugate formation, and polarization of cytotoxic granules is crucial for NK cell-mediated killing. Thus, NK cells from HIV-infected persons may be impaired in their ability to form immune synapses with target cells. Furthermore, the HIV protein Tat was found to inhibit NK cell-mediated lysis by blocking L-type $Ca^{++}$ channels [242]. $Ca^{++}$ infuxes are essential for activation of CAMK-II, rearranging microtubules and triggering degranulation of NK cells following activation of cells via LFA-1 [150]. Furthermore, gp120 binding to CD4 also inhibits LFA-1-mediated cell–cell interactions by causing dissociation of the integrin from its cytoplasmic partner cytohesin [243]. Few studies have been undertaken to investigate functional capabilities of NK cells from these persons for conjugate formation and triggering their cytolytic machinery. It was demonstrated that NK cells from the infected persons may form conjugates with target cells but are defective in triggering their cytolytic mediators onto the target cells [19, 24]. The inability of NK cells from HIV-infected individuals to establish and maintain an effective immune synapse and trigger its cytolytic mediators may represent a fundamental reason for compromised NK cell functions in HIV-infected persons.

It appears that absolute numbers and percentages of NK cells decrease over time in HIV-infected persons. $CD8^+CD16^+$ and $CD56^+CD16^+$ NK cell subsets have been reported to decrease in percentages and in absolute numbers in these individuals. These decreases are often accompanied by the expansion of a functionally defective subset of $CD16^+CD56^-$ NK cells, which express KIR. It is noteworthy that it is the $CD16^-CD56^+$ subset of NK cells that expands in primary viral infections. The changes in NK cells are more severe with the onset of AIDS and correlate with clinical condition of the patients [27, 32, 35, 216]. The decreases in NK cell subsets correlate significantly with depletion of the $CD4^+$ T cells in these patients [14, 16], suggesting that $CD4^+$ T cell-secreted cytokines (e.g., IL-2, IL-21) may be important in vivo in maintaining NK cell survival. Alternatively, the declines in the numbers of these two types of immune cells may reflect immune dysfunction independently of each other. It may be relevant to mention here that $CD56^+$ NK cells develop and differentiate in thymus and secondary lymphoid organs in T cell-rich areas. A progressive destruction of the architecture of these organs as well as depletion of $CD4^+$ T cells in HIV-infected persons may result in depletion of this subset of NK cells. Overall, NK cells from HIV-infected persons express lower levels of perforin and higher levels of SHIP, which may be responsible for their poor cytolytic and activating potentials [58]. As mentioned elsewhere, the envelope glycoproteins of HIV can up-regulate proapoptotic genes and reduce survivability and vigor of NK cells (see the next section). Recombinant gp120 inhibits NK cell functions when added to in vitro microcytotoxicity assays. Furthermore, certain peptides derived from the protein also have NK cell inhibitory properties [80, 244, 245]. The exact mechanism of inhibition of the peptides remains unknown. Finally, stress could be a factor in suppressing NK cell functions in HIV-infected persons. Cortisol has been shown to act in synergism with HIV proteins in mediating the suppressive effects on NK cells [244].

In vitro studies have shown that several exogenous cytokines, e.g., IL-2, IL-12, IL-15, IFN-$\alpha$, etc., increase cytolytic and ADCC effector function of NK cells from HIV-infected individuals. However, the responses were significantly lower in HIV-infected individuals as compared with HIV-seronegative, healthy controls [19, 34, 43, 239, 246]. These observations suggest that NK cells from HIV-infected persons may have decreased expression of cytokine receptors and/or may have defects in cytokine-induced signaling pathways. This may explain why NK cells from these patients produce defective LAK cells when they are incubated with cytokines, e.g., with IL-2 [25].

## EFFECT OF HAART ON ANTIVIRAL NK CELL RESPONSES

HAART suppresses HIV replication to undetectable limits in the circulation of HIV-infected persons. Over time, this leads to improvement in the NK cell functions. However, a prolonged treatment is needed for tangible improvements in the NK cell compartment. In most of cases, the recovery is only partial. NK cells and the receptor expression tend to normalize in the treated persons; however, certain NK cell functions, e.g., their ability to produce IFN-$\gamma$ in response to IL-2 and IL-15, remain compromised [50]. In one study, HAART reversed expression of iKIR on NK cells after 2 years' administration, but the reduced expression of activating receptors persisted [51]. Similarly, a normalizing effect of HAART was observed on the expression of 2B4 on NK cells [56]. HAART, for more than 6 months, caused a differential disappearance of iKIR on virus-specific CTL but usually had no effect on ILT-2 expression [45]. HAART also does not have any effect on the expansion of NKG2C on NK cells and CTL in HIV- and HCMV-coinfected patients. As mentioned earlier, this can be ascribed to the fact that HCMV and not HIV causes expansion of these cells [115, 116, 247]. In primary HIV infection, an early start of HAART may normalize changes in the NK cell compartment within 6

months [61]. The baseline activation of the immune system and viral load determines the extent to which innate immune parameters could be reconstituted by HAART in HIV-infected AIDS patients. Continued viral suppression and reduction in immune activation for more than 1 year resulted in recovery of pDC, better NK/DC interactions, and partial restoration of NK cell numbers and functions [248].

If NK cells become infected, they may act as latent reservoirs for the virus, as the infection could persist in these cells even after years of HAART [219, 221]. Thus, immunotherapy should be considered for invigorating NK cell responses along with chemotherapy.

## NOVEL APPROACHES FOR ENHANCING ANTI-HIV NK CELL RESPONSES

A better understanding of interactions between HIV and NK cell responses of the host has led to novel, rational approaches for boosting antiviral immunity in HIV-infected persons and for designing more effective anti-AIDS vaccines. These approaches are listed in **Table 4** and are discussed in the following sections.

### Blocking inhibitory NKRs

As stated above, KIR, NKG2A, and ILT-2 are the main inhibitory receptors that control NK cell activities in an individual. They are also expressed on a subset of antigen-experienced effector/memory CTL, in which they increase the antigen-mediated activation threshold. Cumulative data have shown that the expression of iKIR increases on NK cells and CTL in HIV-infected persons, especially under viremic conditions [16, 40, 148]. Blocking the functional activities of these receptors with receptor- or MHC-specific antibodies or with small molecular weight inhibitors increases cytolytic activities and cytokine secretion from NK cells and CTL. Studies in animal models have shown that blocking of the inhibitory NKR LY49 in vivo also augments the anti-tumor effects of NK cells and CTL and results in tumor regression [143]. This strategy may boost antiviral effects of NK cells and CTL in HIV-infected individuals. In this regard, in vitro studies have shown that masking of iKIR by mAb increases the cytolytic activities of HIV-specific CTL from HIV-infected patients against autologous, virus-infected cells [40]. The receptors could also be blocked by soluble MHC antigens. However, they are more likely to bind TCR preferentially than KIR, and hence, they may block CTL functions. Small molecular weight chemical compounds could be synthesized to specifically block KIR–MHC interactions. These immunotherapies will have to be tailored individually, as the patients may differ in their KIR–HLA combination repertoires. As the blocking of inhibitory receptors on NK cells and CTL may promote killing of autologous cells and uncontrolled cytokine production, the treatment could cause immunopathology. Furthermore, the strategy may interfere with the development of long-term, virus-specific memory and even may promote apoptosis of these effector/memory cells. Admittedly, such treatments could be risky, and the treated patients will have to be carefully monitored for any untoward effects.

TABLE 4. Novel Ways of Boosting Anti-HIV Adaptive and Innate Immunity

1. By manipulating the activity of NKRs.
2. By adoptive transfer of autologous or alloreactive NK cells.
3. By the adoptive transfer of HLA-C- and HLA-E-restricted, antiviral CTL.
4. Using HLA-C- and -E-restricted, immunogenic viral peptides.
5. Using NK cell activity-enhancing, novel adjuvants.
6. Targeting NK cells to HIV-infected cells.

### Novel ways of anti-HIV cell therapy

In the past, the infusions of the in vitro-expanded, autologous, HIV-specific CTL have been used as immunotherapeutic tools in HIV-infected AIDS patients without much success [249, 250]. A better strategy may involve expansion and infusion of the CTL specific for HLA-C-restricted viral peptides, as the virus does not down-regulate the expression of this MHC antigen in the infected cells. Similarly, in vitro-expanded, lymphokine-activated, autologous NK cell clones that express inhibitory receptors for HLA-A or -B but not for HLA-C or -E could be considered as immunotherapeutic tools in these patients. These cells should kill only HIV-infected cells that have down-regulated HLA-A and -B but not HLA-C or -E. Similarly, lymphokine-treated, heterologous NK cells that express one or more iKIR specific for the recipient HLA-A or -B could also be beneficial. Alloreactive NK cells are known to preferentially kill hematopoietic cells in MHC-disparate recipients without causing graft-versus-host disease (GvHD). The beneficial effects of alloreactive NK cells have been well documented in leukemia patients receiving bone marrow transplants (reviewed in refs. [251, 252]). The potential of alloreactive NK cells as therapeutic tools for viral infections including HIV is worth investigation.

### Selecting epitopes for anti-HIV vaccination

As stated above, HIV-1 differentially down-regulates the expression of MHC class I antigens on the surface of infected cells for evading NK and CTL-mediated killing. The viral protein Nef causes degradation of most of the HLA-A, HLA-B, and CD1d antigens but leaves HLA-C and HLA-E to intact on the cell surface [96–98]. As HLA-C and -E act as ligands for inhibitory receptors on NK cells, the virus-infected cells maintain their resistance to NK cells by maintaining their expression on the surface of infected cells. The virus, in fact, increases the expression of HLA-E by providing a peptide (within the viral protein p24) that can bind to this nonclassical MHC antigen. It is noteworthy that HLA-C is not exclusively used as ligands for NKRs. Several HIV peptides are presented to T cells via this MHC antigen [253–255]. These peptides may serve as better immunogens for inducing anti-HIV CTL, as HLA-C are not degraded from the surface of the virus-infected cells, and therefore, virus may not be able to hide from the peptide-specific CTL. The notion is supported by the reported association between the presence of HLA-C-restricted viral peptides in HIV-infected individuals and their long-term non-progression toward AIDS [256]. Furthermore, the existence of

CTL, which recognize HLA-E-restricted viral peptides, has also been demonstrated for different viruses [257]. We could not find any study in literature about HLA-E-restricted HIV peptides presented to CTL. These HIV peptides could also be considered as immunogens for vaccination against HIV.

It is noteworthy that HLA-A and -B antigens mainly present viral peptides recognized by HIV-specific CTL. Many studies have shown that several "protective" HLA-B allotypes can present broadly reactive, immunodominant peptides to CTL [258, 259]. On the other hand, only a few HLA-C-restricted HIV epitopes have been described [253–255]. In part, it could be a result of the fact that this HLA antigen is expressed at relatively lower levels on human cells [260]. CTL may not be able to detect the peptide-complexed antigen. Using knowledge-based algorithms, Tong et al. [261] have shown that HLA-C-restricted peptides could be found in most HIV proteins. Further studies are needed to evaluate the functional significance of these epitopes.

### Invigorating NK cells with cytokines/anticytokines

Use of cytokines for enhancing innate and adaptive immunity of the host has been a cherished goal of immunologists since the discovery of IL-2 in the early 1980s. However, the toxicities associated with their use have always been prohibitive. Potential cytokines that can be used to enhance NK cell activity in vivo include IL-2, IL-15, IL-21, as well as ligands for c-Kit and FMS-like receptor tyrosine kinases (Flt-3). It may be relevant to mention here that IL-2 and/or IL-2-activated killer cell infusions have not been promising as therapeutic tools in cancer patients [262, 263]. In the context of HIV infection, these immune enhancers may pose another complication. They may increase HIV replication and act as paracrine growth factors in AIDS-related malignancies. In this regard, IL-15 has been shown to be relatively less mitogenic and less toxic and to have minimal effects on HIV replication. Furthermore, it inhibits spontaneous apoptosis in NK cells and CTL from HIV-infected patients by increasing the expression of antiapoptotic protein Bcl-$X_L$ (reviewed in ref. [229]). The cytokine is an absolute necessity for normal development, differentiation, and homeostasis of HNK cells. IL-21 is another relatively recently discovered cytokine produced mainly from activated $CD4^+$ T cells. It increases cytolytic potential of NK cells and is even less mitogenic than IL-15. However, no data are yet available about its effects on HIV replication and cytotoxicity.

### Enhancing immunogenicity of viral immunogens

NK cell activation in the beginning of a viral infection has a strong adjuvant effect. Activated NK cells kill virus-infected cells, whose products send a "danger signal" to the host for initiating antiviral inflammatory and immune responses [264]. The role of NK cell-secreted IFN-γ in this connection has been well documented. The studies about interactions between activated NK cells and DC interactions also testify to the role of NK cells in the generation of adaptive immunity. DC pulsed with tumor cell lysates are effective in mediating anti-tumor immunity in vitro and in vivo in animal models. It has been shown that these DC mediate these adjuvant effects by activating NK cells [265]. It was also demonstrated that the presence of IL-18 in in vitro cultures of NK cells, DC, T cells, and tumor cells leads to rapid generation of tumor-specific CTL [266]. These studies show that activating NK cells at or prior to immunization may lead to effective antiviral immunity. This activation may be achieved by cytokines and/or TLR agonists, which also cause release of cytokines. α-Galactosyl ceramide has also been used as an adjuvant. It is presented by APC via CD1d to NKT cells, which in turn, activate NK cells [267, 268]. Based on our present understanding of NK cell biology, inhibiting KIR–MHC interactions and/or inducing expression of ligands for activating NKRs may produce better adjuvant effects than our currently used adjuvant formulations in vaccination regimens. Indeed, better antigen-specific, immune responses were induced when vectors expressing ligands for NKG2D were used along with immunogen [269]. These novel approaches should contribute to better and more effective vaccine strategies against HIV infection and AIDS.

## PERSPECTIVE AND FUTURE DIRECTIONS

Despite spectacular advances made in understanding NK cell biology, there still remain unknown aspects of these cells, which should be addressed in future research. For example, we are still far from discovering all NKRs. An area that needs immediate attention concerns finding ligands for aKIR and NCR. Furthermore, we need to know better how the NKR repertoire of the host is shaped and what effects the host MHC has in shaping this repertoire. A lot has been learned about interactions between NK cells and DC. It appears that NK cells could also interact directly with T cells. This could be an extremely productive area of research.

It is now evident from several studies that different NKRs, particularly of the inhibitory type (e.g., KIR, ILT, KLR-G1), are expressed frequently on antigen-experienced $CD8^+$ T cells and less frequently on $CD4^+$ T cells. These receptors seem to be expressed at distinct stages in the course of differentiation and development of these cells. They may serve important functions; e.g., they may prevent apoptosis and increase survival of the cells and/or may increase the activation threshold of the effector cells to prevent autoaggression. These receptors could serve as important markers to distinguish different developmental stages of these cells. This knowledge may allow us to identify exact defects, which appear in these cells in viral infections and malignancy.

Activating NKRs of the KIR family have been reported to occur on $CD4^+$ T cells under certain disease conditions. Their expression has been described on CTL in HIV-infected viremic persons [62]. It is not known what triggers their expression and what are the consequences of this expression—how the receptor-positive cells differ from the receptor-negative cells in terms of their proliferation, cytokine production, and interaction with other cells in the body. It would also be of great interest to see if and how HIV induces the expression of their ligands on infected human cells.

In the context of HIV infections, future efforts should be directed at knowing which of the NKRs are aberrantly expressed on the surface of NK cells as well as on other immu-

nocytes, e.g., monocyte/macrophages, DC, and B and T cells ($CD4^+$ and $CD8^+$ subsets). As mAb are not available for all of these receptors, and the ones that exist may not distinguish between the activating and inhibitory forms of these receptors, therefore, one may have to use alternate methods. Fortunately, the NKR genes, which have been studied so far, seem to be regulated at the transcriptional level. This suggests that real-time RT-PCR and/or oligonucleotide microarrays with appropriate controls may give a fair idea of the genes whose expression may be dysregulated in HIV-infected individuals.

As discussed above, modulation of interactions between NKRs and their ligands may represent an important tool of immunotherapy. Studies should be performed in animal models to see the long-term effects of these interventions on the resistance of the host to pathogens and development of tumors. Small, antagonist chemical molecules, peptides, and humanized receptor-specific mAb should be developed for their potential use in boosting innate and adaptive immunity in HIV-infected individuals.

We also need to develop innovative means to target NK cells toward HIV-infected cells. In this connection, fusion proteins combining intracellular $\zeta$ chains with the extracellular region of CD4 or with HIV-specific single-chain antibody have been developed. Transduction of these fusion proteins into primary human NK cells via retroviral vectors redirects their killing toward HIV-infected cells [270]. Another group has made a fusion protein combining gp120-specific antibodies of IgA and IgG isotypes. It is meant to kill HIV-infected cells by linking the viral envelope protein with FcR on NK and other immune cells [271]. Finally, the potential of alloreactive NK cells as therapeutic tools in viral infections, particularly with HIV, is worth exploring. These cells have benefited leukemia patients undergoing bone marrow transplantation and do not seem to cause GvHD (reviewed in ref. [252]). Fortunately, now, the technology exists for obtaining fully differentiated and functional NK cells from human stem cells [272].

Invigorating and activating NK cells may benefit HIV-infected persons in controlling the infection. However, it should not be forgotten that activated NK cells are equipped with a lot of destructive potential. Their excessive activation may cause tissue destruction and contribute toward pathogenesis of the disease. Therefore, NK cell activity-enhancing treatments will have to be closely monitored for undesirable consequences.

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## REFERENCES

1. Alter, G., Malenfant, J. M., Delabre, R. M., Burgett, N. C., Yu, X. G., Lichterfeld, M., Zaunders, J., Altfeld, M. (2004) Increased natural killer cell activity in viremic HIV-1 infection. *J. Immunol.* **173,** 5305–5311.
2. Giavedoni, L. D., Velasquillo, M. C., Parodi, L. M., Hubbard, G. B., Hodara, V. L. (2000) Cytokine expression, natural killer cell activation, and phenotypic changes in lymphoid cells from rhesus macaques during acute infection with pathogenic simian immunodeficiency virus. *J. Virol.* **74,** 1648–1657.
3. Baratin, M., Roetynck, S., Lepolard, C., Falk, C., Sawadogo, S., Uematsu, S., Akira, S., Ryffel, B., Tiraby, J-G., Alexopoulou, L., Kirschning, C. J., Gysin, J., Vivier, E., Ugolini, S. (2005) Natural killer cell and macrophage cooperation in MyD88-dependent innate responses to *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. USA* **102,** 14747–14752.
4. Hart, O. M., Athie-Morales, V., O'Connor, G. M., Gardiner, C. M. (2005) TLR7/8-mediated activation of human NK cells results in accessory cell-dependent IFN-{$\gamma$} production. *J. Immunol.* **175,** 1636–1642.
5. Alter, G., Suscovich, T. J., Teigen, N., Meier, A., Streeck, H., Brander, C., Altfeld, M. (2007) Single-stranded RNA derived from HIV-1 serves as a potent activator of NK cells. *J. Immunol.* **178,** 7658–7666.
6. Fehniger, T. A., Herbein, G., Yu, H., Para, M. I., Bernstein, Z. P., O'Brien, W. A., Caligiuri, M. A. (1998) Natural killer cells from HIV-1+ patients produce C–C chemokines and inhibit HIV-1 infection. *J. Immunol.* **161,** 6433–6438.
7. Kottilil, S., Shin, K., Planta, M., McLaughlin, M., Hallahan, C., Ghany, M., Chun, T., Sneller, M., Fauci, A. (2004) Expression of chemokine and inhibitory receptors on natural killer cells: effect of immune activation and HIV viremia. *J. Infect. Dis.* **189,** 1193–1198.
8. Oliva, A., Kinter, A. L., Vaccarezza, M., Rubbert, A., Catanzaro, A., Moir, S., Monaco, J., Ehler, L., Mizell, S., Jackson, R., Li, Y., Romano, J. W., Fauci, A. S. (1998) Natural killer cells from human immunodeficiency virus (HIV)-infected individuals are an important source of CC-chemokines and suppress HIV-1 entry and replication in vitro. *J. Clin. Invest.* **102,** 223–231.
9. Forthal, D. N., Landucci, G., Daar, E. S. (2001) Antibody from patients with acute human immunodeficiency virus (HIV) infection inhibits primary strains of HIV type 1 in the presence of natural-killer effector cells. *J. Virol.* **75,** 6953–6961.
10. Zhang, D., Shankar, P., Xu, Z., Harnisch, B., Chen, G., Lange, C., Lee, S. J., Valdez, H., Lederman, M. M., Lieberman, J. (2003) Most antiviral CD8 T cells during chronic viral infection do not express high levels of perforin and are not directly cytotoxic. *Blood* **101,** 226–235.
11. Kottilil, S., Chun, T., Moir, S., Liu, S., McLaughlin, M., Hallahan, C., Maldarelli, F., Corey, L., Fauci, A. (2003) Innate immunity in human immunodeficiency virus infection: effect of viremia on natural killer cell function. *J. Infect. Dis.* **187,** 1038–1045.
12. Ravet, S., Scott-Algara, D., Bonnet, E., Tran, H. K., Tran, T., Nguyen, N., Truong, L. X., Theodorou, I., Barre-Sinoussi, F., Pancino, G., Paul, P. (2007) Distinctive NK-cell receptor repertoires sustain high-level constitutive NK-cell activation in HIV-exposed uninfected individuals. *Blood* **109,** 4296–4305.
13. Scott-Algara, D., Truong, L. X., Versmisse, P., David, A., Luong, T. T., Nguyen, N. V., Theodorou, I., Barre-Sinoussi, F., Pancino, G. (2003) Cutting edge: increased NK cell activity in HIV-1-exposed but uninfected Vietnamese intravascular drug users. *J. Immunol.* **171,** 5663–5667.
14. Douglas, S. D., Durako, S. J., Tustin, N. B., Houser, J., Muenz, L., Starr, S. E., Wilson, C. (2001) Natural killer cell enumeration and function in HIV-infected and high-risk uninfected adolescents. *AIDS Res. Hum. Retroviruses* **17,** 543–552.
15. Ironson, G., Balbin, E., Solomon, G., Fahey, J., Klimas, N., Schneiderman, N., Fletcher, M. (2001) Relative preservation of natural killer cell cytotoxicity and number in healthy AIDS patients with low CD4 cell counts. *AIDS* **15,** 2065–2073.
16. Ahmad, R., Sindhu, S., Tran, P., Toma, E., Morisset, R., Menezes, J., Ahmad, A. (2001) Modulation of expression of the MHC class I-binding natural killer cell receptors, and NK activity in relation to viral load in HIV-infected/AIDS patients. *J. Med. Virol.* **65,** 431–440.
17. Heeney, J. L., Plotkin, S. A. (2006) Immunological correlates of protection from HIV infection and disease. *Nat. Immunol.* **7,** 1281–1284.
18. Rutjens, E., Mazza, S., Biassoni, R., Koopman, G., Radic, L., Fogli, M., Costa, P., Mingari, M. C., Moretta, L., Heeney, J., De Maria, A. (2007) Differential NKp30 inducibility in chimpanzee NK cells and conserved NK cell phenotype and function in long-term HIV-1-infected animals. *J. Immunol.* **178,** 1702–1712.
19. Bonavida, B., Katz, J., Gottlieb, M. (1986) Mechanism of defective NK cell activity in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. I. Defective trigger on NK cells for NKCF production by target cells, and partial restoration by IL 2. *J. Immunol.* **137,** 1157–1163.
20. Zaizov, R., Cohen, I., Luria, D., Trainin, N., Umiel, T. (1986) In vitro restoration by interleukin-2 (IL-2) of the impaired natural killer cell

activities, IL-2 receptor expression, and T cell proliferation in hemophilia. *J. Biol. Response Mod.* **5,** 339–350.
21. Ruscetti, F. W., Mikovits, J. A., Kalyanaraman, V. S., Overton, R., Stevenson, H., Stromberg, K., Herberman, R. B., Farrar, W. L., Ortaldo, J. R. (1986) Analysis of effector mechanisms against HTLV-I- and HTLV-III/LAV-infected lymphoid cells. *J. Immunol.* **136,** 3619–3624.
22. Katz, J. D., Mitsuyasu, R., Gottlieb, M., Lebow, L., Bonavida, B. (1987) Mechanism of defective NK cell activity in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. II. Normal antibody-dependent cellular cytotoxicity (ADCC) mediated by effector cells defective in natural killer (NK) cytotoxicity. *J. Immunol.* **139,** 55–60.
23. Plaeger-Marshall, S., Spina, C., Giorgi, J., Mitsuyasu, R., Wolfe, P., Gottlieb, M., Beall, G. (1987) Alterations in cytotoxic and phenotypic subsets of natural killer cells in acquired immune deficiency syndrome (AIDS). *J. Clin. Immunol.* **7,** 16–23.
24. Sirianni, M. C., Soddu, S., Malorni, W., Arancia, G., Aiuti, F. (1988) Mechanism of defective natural killer cell activity in patients with AIDS is associated with defective distribution of tubulin. *J. Immunol.* **140,** 2565–2568.
25. Gryllis, C., Wainberg, M., Gornitsky, M., Brenner, B. (1990) Diminution of inducible lymphokine-activated killer cell activity in individuals with AIDS-related disorders. *AIDS* **4,** 1205–1212.
26. Tyler, D. S., Stanley, S. D., Nastala, C. A., Austin, A. A., Bartlett, J. A., Stine, K. C., Lyerly, H. K., Bolognesi, D. P., Weinhold, K. J. (1990) Alterations in antibody-dependent cellular cytotoxicity during the course of HIV-1 infection. Humoral and cellular defects. *J. Immunol.* **144,** 3375–3384.
27. Mansour, I., Doinel, C., Rouger, P. (1990) CD16+ NK cells decrease in all stages of HIV infection through a selective depletion of the CD16+CD8+CD3– subset. *AIDS Res. Hum. Retroviruses* **6,** 1451–1457.
28. Scott-Algara, D., Vuillier, F., Cayota, A., Dighiero, G. (1992) Natural killer (NK) cell activity during HIV infection: a decrease in NK activity is observed at the clonal level and is not restored after in vitro long-term culture of NK cells. *Clin. Exp. Immunol.* **90,** 181–187.
29. Cauda, R., Goletti, D., Lucia, M. B., Tumbarello, M., Rumi, C., Orengo, A. M., Moretta, A. (1994) Analysis of natural killer (NK) cell subsets defined by the expression of two novel surface antigens (EB6 and GL183) in AIDS and AIDS-related conditions. *Clin. Immunol. Immunopathol.* **70,** 198–205.
30. Brenner, B. G., Gornitsky, M., Wainberg, M. A. (1994) Interleukin-2-inducible natural immune (lymphokine-activated killer cell) responses as a functional correlate of progression to AIDS. *Clin. Diagn. Lab. Immunol.* **1,** 538–544.
31. Sirianni, M. C., Mezzaroma, I., Aiuti, F., Moretta, A. (1994) Analysis of the cytolytic activity mediated by natural killer cells from acquired immunodeficiency syndrome patients in response to phytohemagglutinin or anti-CD16 monoclonal antibody. *Eur. J. Immunol.* **24,** 1874–1878.
32. Voiculescu, C., Avramescu, C., Balasoiu, M., Turculeanu, A., Radu, E. (1994) Changes of blood CD16/CD56 (NK) and HLA-DR/CD3-positive lymphocyte amounts in HIV-infected children, as related to clinical progression and p24-antigen/p24-antibody presence. *FEMS Immunol. Med. Microbiol.* **9,** 217–221.
33. Ahmad, A., Morisset, R., Thomas, R., Menezes, J. (1994) Evidence for a defect of antibody-dependent cellular cytotoxic (ADCC) effector function and anti-HIV gp120/41-specific ADCC-mediating antibody titres in HIV-infected individuals. *J. Acquir. Immune Defic. Syndr.* **7,** 428–437.
34. Ullum, H., Gøtzsche, P., Victor, J., Dickmeiss, E., Skinhøj, P., Pedersen, B. (1995) Defective natural immunity: an early manifestation of human immunodeficiency virus infection. *J. Exp. Med.* **182,** 789–799.
35. Hu, P. F., Hultin, L., Hultin, P., Hausner, M., Hirji, K., Jewett, A., Bonavida, B., Detels, R., Giorgi, J. (1995) Natural killer cell immunodeficiency in HIV disease is manifest by profoundly decreased numbers of CD16+CD56+ cells and expansion of a population of CD16dimCD56– cells with low lytic activity. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* **10,** 331–340.
36. Lucia, B., Jennings, C., Cauda, R., Ortona, L., Landay, A. (1995) Evidence of a selective depletion of a CD16+ CD56+ CD8+ natural killer cell subset during HIV infection. *Cytometry* **22,** 10–15.
37. Ahmad, A., Menezes, J. (1996) Defective killing activity against gp120/41-expressing human erythroleukaemic K562 cell line by monocytes and natural killer cells from HIV-infected individuals. *AIDS* **10,** 143–149.
38. Bruunsgaard, H., Pedersen, C., Skinhøj, P., Pedersen, B. (1997) Clinical progression of HIV infection: role of NK cells. *Scand. J. Immunol.* **46,** 91–95.
39. Zerhouni, B., Sanhadji, K., Kehrli, L., Livrozet, J., Touraine, J. (1997) Interleukin (IL)-2 deficiency aggravates the defect of natural killer cell activity in AIDS patients. *Thymus* **24,** 147–156.
40. De Maria, A., Ferraris, A., Guastella, M., Pilia, S., Cantoni, C., Polero, L., Mingari, M. C., Bassetti, D., Fauci, A. S., Moretta, L. (1997) Expression of HLA class I-specific inhibitory natural killer cell receptors in HIV-specific cytolytic T lymphocytes: impairment of specific cytolytic functions. *Proc. Natl. Acad. Sci. USA* **94,** 10285–10288.
41. Galiani, M. D., Aguado, E., Tarazona, R., Romero, P., Molina, I., Santamaria, M., Solana, R., Pena, J. (1999) Expression of killer inhibitory receptors on cytotoxic cells from HIV-1-infected individuals. *Clin. Exp. Immunol.* **115,** 472–476.
42. Andre, P., Brunet, C., Guia Hervé, S., Sampol, G., Vivier, E., Dignat-George, F. (1999) Differential regulation of killer cell Ig-like receptors and CD94 lectin-like dimers on NK and T lymphocytes from HIV-1-infected individuals. *Eur. J. Immunol.* **29,** 1076–1085.
43. Ullum, H., Cozzi Lepri, A., Aladdin, H., Katzenstein, T., Victor, J., Phillips, A., Gerstoft, J., Skinhøj, P., Klarlund Pedersen, B. (1999) Natural immunity and HIV disease progression. *AIDS* **13,** 557–563.
44. Geertsma, M. F., Stevenhagen, A., van Dam, E., Nibbering, P. (1999) Expression of ζ molecules is decreased in NK cells from HIV-infected patients. *FEMS Immunol. Med. Microbiol.* **26,** 249–257.
45. Costa, P., Rusconi, S., Mavilio, D., Fogli, M., Murdaca, G., Pende, D., Mingari, M., Galli, M., Moretta, L., De Maria, A. (2001) Differential disappearance of inhibitory natural killer cell receptors during HAART and possible impairment of HIV-1-specific CD8 cytotoxic T lymphocytes. *AIDS* **15,** 965–974.
46. Tarazona, R., Casado, J. G., Delarosa, O., Torre-Cisneros, J., Villanueva, J. L., Sanchez, B., Galiani, M. D., Gonzalez, R., Solana, R., Pena, J. (2002) Selective depletion of CD56dim NK cell subsets and maintenance of CD56bright NK cells in treatment-naive HIV-1-seropositive individuals. *J. Clin. Immunol.* **22,** 176–183.
47. Barboza, J. M., Salmen, S., Cova, J. A., Albarran, B., Goncalves, L., Borges, L., Hernandez, M., Berrueta, L. (2002) Uncoupling activation-induced modulation of CD16 and CD69 in CD56+ cells during AIDS. *APMIS* **110,** 415–422.
48. Parato, K. G., Kumar, A., Badley, A., Sanchez-Dardon, J., Chambers, K., Young, C., Lim, W., Kravcik, S., Cameron, D., Angel, J. (2002) Normalization of natural killer cell function and phenotype with effective anti-HIV therapy and the role of IL-10. *AIDS* **16,** 1251–1256.
49. De Maria, A., Fogli, M., Costa, P., Murdaca, G., Puppo, F., Mavilio, D., Moretta, A., Moretta, L. (2003) The impaired NK cell cytolytic function in viremic HIV-1 infection is associated with a reduced surface expression of natural cytotoxicity receptors (NKp46, NKp30 and NKp44). *Eur. J. Immunol.* **33,** 2410–2418.
50. Goodier, M. R., Imami, N., Moyle, G., Gazzard, B., Gotch, F. (2003) Loss of the CD56hiCD16– NK cell subset and NK cell interferon-γ production during antiretroviral therapy for HIV-1: partial recovery by human growth hormone. *Clin. Exp. Immunol.* **134,** 470–476.
51. Mavilio, D., Benjamin, J., Daucher, M., Lombardo, G., Kottilil, S., Planta, M. A., Marcenaro, E., Bottino, C., Moretta, L., Moretta, A., Fauci, A. S. (2003) Natural killer cells in HIV-1 infection: dichotomous effects of viremia on inhibitory and activating receptors and their functional correlates. *Proc. Natl. Acad. Sci. USA* **100,** 15011–15016.
52. Fogli, M., Costa, P., Murdaca, G., Setti, M., Mingari, M., Moretta, L., Moretta, A., De Maria, A. (2004) Significant NK cell activation associated with decreased cytolytic function in peripheral blood of HIV-1-infected patients. *Eur. J. Immunol.* **34,** 2313–2321.
53. Mela, C. M., Burton, C., Imami, N., Nelson, M., Steel, A., Gazzard, B., Gotch, F., Goodier, M. (2005) Switch from inhibitory to activating NKG2 receptor expression in HIV-1 infection: lack of reversion with highly active antiretroviral therapy. *AIDS* **19,** 1761–1769.
54. Mavilio, D., Lombardo, G., Benjamin, J., Kim, D., Follman, D., Marcenaro, E., O'Shea, M. A., Kinter, A., Kovacs, C., Moretta, A., Fauci, A. S. (2005) Characterization of CD56–/CD16+ natural killer (NK) cells: a highly dysfunctional NK subset expanded in HIV-infected viremic individuals. *Proc. Natl. Acad. Sci. USA* **102,** 2886–2891.
55. Martini, F., Agrati, C., D'Offizi, G., Poccia, F. (2005) HLA-E upregulation induced by HIV infection may directly contribute to CD94-mediated impairment of NK cells. *Int. J. Immunopathol. Pharmacol.* **18,** 269–276.
56. Ostrowski, S. R., Ullum, H., Pedersen, B. K., Gerstoft, J., Katzenstein, T. L. (2005) 2B4 expression on natural killer cells increases in HIV-1 infected patients followed prospectively during highly active antiretroviral therapy. *Clin. Exp. Immunol.* **141,** 526–533.
57. Alter, G., Teigen, N., Davis, B. T., Addo, M. M., Suscovich, T. J., Waring, M. T., Streeck, H., Johnston, M. N., Staller, K. D., Zaman, M. T., Yu, X. G., Lichtefeld, M., Basgoz, N., Rosenberg, E. S., Altfeld, M. (2005) Sequential deregulation of NK cell subset distribution and function starting in acute HIV-1 infection. *Blood* **106,** 3366–3369.

58. Alter, G., Suscovich, T., Kleyman, M., Teigen, N., Streeck, H., Zaman, M., Meier, A., Altfeld, M. (2006) Low perforin and elevated SHIP-1 expression is associated with functional anergy of natural killer cells in chronic HIV-1 infection. *AIDS* **20,** 1549–1551.
59. Wilson, T. J., Presti, R. M., Tassi, I., Overton, E. T., Cella, M., Colonna, M. (2007) FcRL6, a new ITIM-bearing receptor on cytolytic cells, is broadly expressed by lymphocytes following HIV-1 infection. *Blood* **109,** 3786–3793.
60. Alter, G., Teigen, N., Ahern, R., Streeck, H., Meier, A., Rosenberg, E. S., Altfeld, M. (2007) Evolution of innate and adaptive effector cell functions during acute HIV-1 infection. *J. Infect. Dis.* **195,** 1452–1460.
61. Titanji, K., Sammicheli, S., De Milito, A., Mantegani, P., Fortis, C., Berg, L., Kärre, K., Travi, G., Tassandin, C., Lopalco, L., Rethi, B., Tambussi, G., Chiodi, F. (2008) Altered distribution of natural killer cell subsets identified by CD56, CD27 and CD70 in primary and chronic human immunodeficiency virus-1 infection. *Immunology* **123,** 164–170.
62. Pascal, V., Yamada, E., Martin, M. P., Alter, G., Altfeld, M., Metcalf, J. A., Baseler, M. W., Adelsberger, J. W., Carrington, M., Anderson, S. K., McVicar, D. W. (2007) Detection of KIR3DS1 on the cell surface of peripheral blood NK cells facilitates identification of a novel null allele and assessment of KIR3DS1 expression during HIV-1 infection. *J. Immunol.* **179,** 1625–1633.
63. Ahmad, A., Menezes, J. (1996) Antibody-dependent cellular cytotoxicity in HIV infections. *FASEB J.* **10,** 258–266.
64. Iannello, A., Ahmad, A. (2005) Role of antibody-dependent cell-mediated cytotoxicity in the efficacy of therapeutic anti-cancer monoclonal antibodies. *Cancer Metastasis Rev.* **24,** 487–499.
65. Deaglio, S., Zubiaur, M., Gregorini, A., Bottarel, F., Ausiello, C. M., Dianzani, U., Sancho, J., Malavasi, F. (2002) Human CD38 and CD16 are functionally dependent and physically associated in natural killer cells. *Blood* **99,** 2490–2498.
66. Khayat, D., Soubrane, C., Andrieu, J., Visonneau, S., Eme, D., Tourani, J., Beldjord, K., Weil, M., Fernandez, E., Jacquillat, C. (1990) Changes of soluble CD16 levels in serum of HIV-infected patients: correlation with clinical and biologic prognostic factors. *J. Infect. Dis.* **161,** 430–435.
67. Ernst, L. K., Metes, D., Herberman, R., Morel, P. (2002) Allelic polymorphisms in the FcγRIIC gene can influence its function on normal human natural killer cells. *J. Mol. Med.* **80,** 248–257.
68. Orange, J. S. (2002) Human natural killer cell deficiencies and susceptibility to infection. *Microbes Infect.* **4,** 1545–1558.
69. Takeuchi, T., Nakagawa, T., Ikemoto, T., Sasaki, M., Makino, S., Shimizu, A., Ohsawa, N. (1999) A novel mutation in the FcγRIIIA gene (CD16) results in active natural killer cells lacking CD16. *Autoimmunity* **31,** 265–271.
70. Wu, J., Edberg, J. C., Redecha, P. B., Bansal, V., Guyre, P. M., Coleman, K., Salmon, J. E., Kimberly, R. P. (1997) A novel polymorphism of Fcγ RIIIa (CD16) alters receptor function and predisposes to autoimmune disease. *J. Clin. Invest.* **100,** 1059–1070.
71. Forthal, D. N., Landucci, G., Bream, J., Jacobson, L. P., Phan, T. B., Montoya, B. (2007) Fc{γ}RIIa genotype predicts progression of HIV infection. *J. Immunol.* **179,** 7916–7923.
72. Hober, D., Jewett, A., Bonavida, B. (1995) Lysis of uninfected HIV-1 gp120-coated peripheral blood-derived T lymphocytes by monocyte-mediated antibody-dependent cellular cytotoxicity. *FEMS Immunol. Med. Microbiol.* **10,** 83–92.
73. Lyerly, H. K., Matthews, T. J., Langlois, A. J., Bolognesi, D. P., Weinhold, K. J. (1987) Human T-cell lymphotropic virus IIIB glycoprotein (gp120) bound to CD4 determinants on normal lymphocytes and expressed by infected cells serves as target for immune attack. *Proc. Natl. Acad. Sci. USA* **84,** 4601–4605.
74. Skowron, G., Cole, B., Zheng, D., Accetta, G., Yen-Lieberman, B. (1997) gp120-directed antibody-dependent cellular cytotoxicity as a major determinant of the rate of decline in CD4 percentage in HIV-1 disease. *AIDS* **11,** 1807–1814.
75. Ahmad, R., Sindhu, S. T. A. K., Toma, E., Morisset, R., Vincelette, J., Menezes, J., Ahmad, A. (2001) Evidence for a correlation between antibody-dependent cellular cytotoxicity-mediating anti-HIV-1 antibodies and prognostic predictors of HIV infection. *J. Clin. Immunol.* **21,** 227–233.
76. Baum, L. L., Cassutt, K. J., Knigge, K., Khattri, R., Margolick, J., Rinaldo, C., Kleeberger, C. A., Nishanian, P., Henrard, D. R., Phair, J. (1996) HIV-1 gp120-specific antibody-dependent cell-mediated cytotoxicity correlates with rate of disease progression. *J. Immunol.* **157,** 2168–2173.
77. Ljunggren, K., Bottiger, B., Biberfeld, G., Karlson, A., Fenyo, E. M., Jondal, M. (1987) Antibody-dependent cellular cytotoxicity-inducing antibodies against human immunodeficiency virus. Presence at different clinical stages. *J. Immunol.* **139,** 2263–2267.
78. Banks, N. D., Kinsey, N., Clements, J., Hildreth, J. (2002) Sustained antibody-dependent cell-mediated cytotoxicity (ADCC) in SIV-infected macaques correlates with delayed progression to AIDS. *AIDS Res. Hum. Retroviruses* **18,** 1197–1205.
79. Brenner, B. G., Dascal, A., Margolese, R. G., Wainberg, M. A. (1989) Natural killer cell function in patients with acquired immunodeficiency syndrome and related diseases. *J. Leukoc. Biol.* **46,** 75–83.
80. Ahmad, A., Ahmad, R. (2003) HIV evasion of host NK cell response and novel ways of its countering and boosting anti-HIV immunity. *Curr. HIV Res.* **1,** 295–307.
81. Bryceson, Y. T., March, M. E., Ljunggren, H-G., Long, E. O. (2006) Activation, coactivation, and costimulation of resting human natural killer cells. *Immunol. Rev.* **214,** 73–91.
82. Heidenreich, F., Arendt, G., Jander, S., Jablonowski, H., Stoll, G. (1994) Serum and cerebrospinal fluid levels of soluble intercellular adhesion molecule 1 (sICAM-1) in patients with HIV-1 associated neurological diseases. *J. Neuroimmunol.* **52,** 117–126.
83. Ward, J., Bonaparte, M., Sacks, J., Guterman, J., Fogli, M., Mavilio, D., Barker, E. (2007) HIV modulates the expression of ligands important in triggering natural killer cell cytotoxic responses on infected primary T-cell blasts. *Blood* **110,** 1207–1214.
84. Sapir, T., Shoenfeld, Y. (2005) Facing the enigma of immunomodulatory effects of intravenous immunoglobulin. *Clin. Rev. Allergy Immunol.* **29,** 185–199.
85. Tha-In, T., Metselaar, H. J., Tilanus, H. W., Groothuismink, Z. M. A., Kuipers, E. J., de Man, R. A., Kwekkeboom, J. (2007) Intravenous immunoglobulins suppress T-cell priming by modulating the bi-directional interaction between dendritic cells and natural killer cells. *Blood* **110,** 3253–3262.
86. Huber, M., Trkola, A. (2007) Humoral immunity to HIV-1: neutralization and beyond. *J. Intern. Med.* **262,** 5–25.
87. Hessell, A. J., Hangartner, L., Hunter, M., Havenith, C. E. G., Beurskens, F. J., Bakker, J. M., Lanigan, C. M. S., Landucci, G., Forthal, D. N., Parren, P. W. H. I., Marx, P. A., Burton, D. R. (2007) Fc receptor but not complement binding is important in antibody protection against HIV. *Nature* **449,** 101–104.
88. Mascola, J. R. (2007) HIV/AIDS: allied responses. *Nature* **449,** 29–30.
89. Montefiori, D. C. (2005) Neutralizing antibodies take a swipe at HIV in vivo. *Nat. Med.* **11,** 593–594.
90. Trkola, A., Kuster, H., Rusert, P., Joos, B., Fischer, M., Leemann, C., Manrique, A., Huber, M., Rehr, M., Oxenius, A., Weber, R., Stiegler, G., Veelar, B., Katinger, H., Aceto, L., Gunthard, H. F. (2005) Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. *Nat. Med.* **11,** 615–622.
91. Brutkiewicz, R. R., Welsh, R. M. (1995) Major histocompatibility complex class I antigens and the control of viral infections by natural killer cells. *J. Virol.* **69,** 3967–3971.
92. Iannello, A., Debbeche, O., Martin, E., Attalah, L. H., Samarani, S., Ahmad, A. (2006) Viral strategies for evading antiviral cellular immune responses of the host. *J. Leukoc. Biol.* **79,** 16–35.
93. Tortorella, D., Gewurz, B. E., Furman, M. H., Schust, D. J., Ploegh, H. L. (2000) Viral subversion of the immune system. *Annu. Rev. Immunol.* **18,** 861–926.
94. Carroll, I. R., Wang, J., Howcroft, T. K., Singer, D. S. (1998) Hiv Tat represses transcription of the [β]2-microglobulin promoter. *Mol. Immunol.* **35,** 1171–1178.
95. Kerkau, T., Bacik, I., Bennink, J. R., Yewdell, J. W., Hunig, T., Schimpl, A., Schubert, U. (1997) The human immunodeficiency virus type 1 (HIV-1) Vpu protein interferes with an early step in the biosynthesis of major histocompatibility complex (MHC) class I molecules. *J. Exp. Med.* **185,** 1295–1306.
96. Bonaparte, M. I., Barker, E. (2004) Killing of human immunodeficiency virus-infected primary T-cell blasts by autologous natural killer cells is dependent on the ability of the virus to alter the expression of major histocompatibility complex class I molecules. *Blood* **104,** 2087–2094.
97. Cohen, G. B., Gandhi, R. T., Davis, D. M., Mandelboim, O., Chen, B. K., Strominger, J. L., Baltimore, D. (1999) The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. *Immunity* **10,** 661–671.
98. Collins, K. L., Baltimore, D. (1999) HIV's evasion of the cellular immune response. *Immunol. Rev.* **168,** 65–74.
99. Bonaparte, M. I., Barker, E. (2003) Inability of natural killer cells to destroy autologous HIV-infected T lymphocytes. *AIDS* **17,** 487–494.
100. Nattermann, J., Nischalke, H., Hofmeister, V., Kupfer, B., Ahlenstiel, G., Feldmann, G., Rockstroh, J., Weiss, E., Sauerbruch, T., Spengler, U.

(2005) HIV-1 infection leads to increased HLA-E expression resulting in impaired function of natural killer cells. *Antivir. Ther.* **10,** 95–107.
101. Derrien, M., Pizzato, N., Dolcini, G., Menu, E., Chaouat, G., Lenfant, F., Barre-Sinoussi, F., Bouteiller, P. L. (2004) Human immunodeficiency virus 1 downregulates cell surface expression of the non-classical major histocompatibility class I molecule HLA-G1. *J. Gen. Virol.* **85,** 1945–1954.
102. Cabello, A., Rivero, A., Garcia, M. J., Lozano, J. M., Torre-Cisneros, J., Gonzalez, R., Duenas, G., Galiani, M. D., Camacho, A., Santamaria, M., Solana, R., Montero, C., Kindelan, J. M., Pena, J. (2003) HAART induces the expression of HLA-G on peripheral monocytes in HIV-1 infected individuals. *Hum. Immunol.* **64,** 1045–1049.
103. Lozano, J. M., González, R., Kindelán, J., Rouas-Freiss, N., Caballos, R., Dausset, J., Carosella, E., Peña, J. (2002) Monocytes and T lymphocytes in HIV-1-positive patients express HLA-G molecule. *AIDS* **16,** 347–351.
104. O'Callaghan, C. A., Bell, J. (1998) Structure and function of the human MHC class Ib molecules HLA-E, HLA-F and HLA-G. *Immunol. Rev.* **163,** 129–138.
105. Vieillard, V., Strominger, J. L., Debre, P. (2005) NK cytotoxicity against CD4+ T cells during HIV-1 infection: a gp41 peptide induces the expression of an NKp44 ligand. *Proc. Natl. Acad. Sci. USA* **102,** 10981–10986.
106. Dunn, C., Chalupny, N. J., Sutherland, C. L., Dosch, S., Sivakumar, P. V., Johnson, D. C., Cosman, D. (2003) Human cytomegalovirus glycoprotein UL16 causes intracellular sequestration of NKG2D ligands, protecting against natural killer cell cytotoxicity. *J. Exp. Med.* **197,** 1427–1439.
107. Chalupny, N. J., Rein-Weston, A., Dosch, S., Cosman, D. (2006) Down-regulation of the NKG2D ligand MICA by the human cytomegalovirus glycoprotein UL142. *Biochem. Biophys. Res. Commun.* **346,** 175–181.
108. Salih, H. R., Antropius, H., Gieseke, F., Lutz, S. Z., Kanz, L., Rammensee, H-G., Steinle, A. (2003) Functional expression and release of ligands for the activating immunoreceptor NKG2D in leukemia. *Blood* **102,** 1389–1396.
109. Salih, H. R., Goehlsdorf, D., Steinle, A. (2006) Release of MICB molecules by tumor cells: mechanism and soluble MICB in sera of cancer patients. *Hum. Immunol.* **67,** 188–195.
110. Cerboni, C., Neri, F., Casartelli, N., Zingoni, A., Cosman, D., Rossi, P., Santoni, A., Doria, M. (2007) Human immunodeficiency virus 1 Nef protein downmodulates the ligands of the activating receptor NKG2D and inhibits natural killer cell-mediated cytotoxicity. *J. Gen. Virol.* **88,** 242–250.
111. Gasser, S., Raulet, D. H. (2006) Activation and self-tolerance of natural killer cells. *Immunol. Rev.* **214,** 130–142.
112. Schrofelbauer, B., Hakata, Y., Landau, N. R. (2007) HIV-1 Vpr function is mediated by interaction with the damage-specific DNA-binding protein DDB1. *Proc. Natl. Acad. Sci. USA* **104,** 4130–4135.
113. Eger, K. A., Unutmaz, D. (2004) Perturbation of natural killer cell function and receptors during HIV infection. *Trends Microbiol.* **12,** 301–303.
114. McMahon, C. W., Raulet, D. H. (2001) Expression and function of NK cell receptors in CD8+ T cells. *Curr. Opin. Immunol.* **13,** 465–470.
115. Guma, M., Cabrera, C., Erkizia, I., Bofill, M., Clotet, B., Ruiz, L., Lopez-Botet, M. (2006) Human cytomegalovirus infection is associated with increased proportions of NK cells that express the CD94/NKG2C receptor in aviremic HIV-1-positive patients. *J. Infect. Dis.* **194,** 38–41.
116. Guma, M., Budt, M., Saez, A., Brckalo, T., Hengel, H., Angulo, A., Lopez-Botet, M. (2006) Expansion of CD94/NKG2C+ NK cells in response to human cytomegalovirus-infected fibroblasts. *Blood* **107,** 3624–3631.
117. Tomasec, P., Braud, V. M., Rickards, C., Powell, M. B., McSharry, B. P., Gadola, S., Cerundolo, V., Borysiewicz, L. K., McMichael, A. J., Wilkinson, G. W. (2000) Surface expression of HLA-E, an inhibitor of natural killer cells, enhanced by human cytomegalovirus gpUL40. *Science* **287,** 1031.
118. Robain, M., Boufassa, F., Hubert, J., Persoz, A., Burgard, M., Meyer, L., SEROCO/HEMOCO Study Groups (2001) Cytomegalovirus seroconversion as a cofactor for progression to AIDS. *AIDS* **15,** 251–256.
119. Borrego, F., Ulbrecht, M., Weiss, E. H., Coligan, J. E., Brooks, A. G. (1998) Recognition of human histocompatibility leukocyte antigen (HLA)-E complexed with HLA class I signal sequence-derived peptides by CD94/NKG2 confers protection from natural killer cell-mediated lysis. *J. Exp. Med.* **187,** 813–818.
120. Pittet, M. J., Speiser, D. E., Valmori, D., Cerottini, J-C., Romero, P. (2000) Cutting edge: cytolytic effector function in human circulating CD8+ T cells closely correlates with CD56 surface expression. *J. Immunol.* **164,** 1148–1152.
121. Andersson, J., Kinloch, S., Sönnerborg, A., Nilsson, J., Fehniger, T., Spetz, A., Behbahani, H., Goh, L., McDade, H., Gazzard, B., Stellbrink, H., Cooper, D., Perrin, L. (2002) Low levels of perforin expression in CD8+ T lymphocyte granules in lymphoid tissue during acute human immunodeficiency virus type 1 infection. *J. Infect. Dis.* **185,** 1355–1358.
122. Allen, J. B., Wong, H., Guyre, P., Simon, G., Wahl, S. (1991) Association of circulating receptor Fc γ RIII-positive monocytes in AIDS patients with elevated levels of transforming growth factor-β. *J. Clin. Invest.* **87,** 1773–1779.
123. Ellery, P. J., Tippett, E., Chiu, Y-L., Paukovics, G., Cameron, P. U., Solomon, A., Lewin, S. R., Gorry, P. R., Jaworowski, A., Greene, W. C., Sonza, S., Crowe, S. M. (2007) The CD16+ monocyte subset is more permissive to infection and preferentially harbors HIV-1 in vivo. *J. Immunol.* **178,** 6581–6589.
124. Focosi, D., Petrini, M. (2007) CD57 expression on lymphoma microenvironment as a new prognostic marker related to immune dysfunction. *J. Clin. Oncol.* **25,** 1289–1291.
125. Hoji, A., Connolly, N. C., Buchanan, W. G., Rinaldo Jr., C. R. (2007) CD27 and CD57 expression reveals atypical differentiation of human immunodeficiency virus type 1-specific memory CD8+ T cells. *Clin. Vaccine Immunol.* **14,** 74–80.
126. Day, C. L., Kaufmann, D. E., Kiepiela, P., Brown, J. A., Moodley, E. S., Reddy, S., Mackey, E. W., Miller, J. D., Leslie, A. J., DePierres, C., Mncube, Z., Duraiswamy, J., Zhu, B., Eichbaum, Q., Altfeld, M., Wherry, E. J., Coovadia, H. M., Goulder, P. J. R., Klenerman, P., Ahmed, R., Freeman, G. J., Walker, B. D. (2006) PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* **443,** 350–354.
127. Trautmann, L., Janbazian, L., Chomont, N., Said, E. A., Gimmig, S., Bessette, B., Boulassel, M-R., Delwart, E., Sepulveda, H., Balderas, R. S., Routy, J-P., Haddad, E. K., Sekaly, R-P. (2006) Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. *Nat. Med.* **12,** 1198–1202.
128. Appay, V., Almeida, J. R., Sauce, D., Autran, B., Papagno, L. (2007) Accelerated immune senescence and HIV-1 infection. *Exp. Gerontol.* **42,** 432–437.
129. Ibegbu, C. C., Xu, Y-X., Harris, W., Maggio, D., Miller, J. D., Kourtis, A. P. (2005) Expression of killer cell lectin-like receptor G1 on antigen-specific human CD8+ T lymphocytes during active, latent, and resolved infection and its relation with CD57. *J. Immunol.* **174,** 6088–6094.
130. Uhrberg, M., Valiante, N. M., Young, N. T., Lanier, L. L., Phillips, J. H., Parham, P. (2001) The repertoire of killer cell Ig-like receptor and CD94:NKG2A receptors in T cells: clones sharing identical α β TCR rearrangement express highly diverse killer cell Ig-like receptor patterns. *J. Immunol.* **166,** 3923–3932.
131. Gunturi, A., Berg, R. E., Forman, J. (2003) Preferential survival of CD8 T and NK cells expressing high levels of CD94. *J. Immunol.* **170,** 1737–1745.
132. Ugolini, S., Vivier, E. (2000) Regulation of T cell function by NK cell receptors for classical MHC class I molecules. *Curr. Opin. Immunol.* **12,** 295–300.
133. Anfossi, N., Doisne, J-M., Peyrat, M-A., Ugolini, S., Bonnaud, O., Bossy, D., Pitard, V., Merville, P., Moreau, J-F., Delfraissy, J-F., Dechanet-Merville, J., Bonneville, M., Venet, A., Vivier, E. (2004) Coordinated expression of Ig-like inhibitory MHC class I receptors and acquisition of cytotoxic function in human CD8+ T cells. *J. Immunol.* **173,** 7223–7229.
134. Roger, J., Chalifour, A., Lemieux, S., Duplay, P. (2001) Cutting edge: Ly49A inhibits TCR/CD3-induced apoptosis and IL-2 secretion. *J. Immunol.* **167,** 6–10.
135. Ugolini, S., Arpin, C., Anfossi, N., Walzer, T., Cambiaggi, A., Forster, R., Lipp, M., Toes, R. E. M., Melief, C. J., Marvel, J., Vivier, E. (2001) Involvement of inhibitory NKRs in the survival of a subset of memory-phenotype CD8+ T cells. *Nat. Immunol.* **2,** 430–435.
136. Young, N. T., Uhrberg, M., Phillips, J. H., Lanier, L. L., Parham, P. (2001) Differential expression of leukocyte receptor complex-encoded Ig-like receptors correlates with the transition from effector to memory CTL. *J. Immunol.* **166,** 3933–3941.
137. Mingari, M. C., Schiavetti, F., Ponte, M., Vitale, C., Maggi, E., Romagnani, S., Demarest, J., Pantaleo, G., Fauci, A. S., Moretta, L. (1996) Human CD8+ T lymphocyte subsets that express HLA class I-specific inhibitory receptors represent oligoclonally or monoclonally expanded cell populations. *Proc. Natl. Acad. Sci. USA* **93,** 12433–12438.
138. Robbins, S. H., Terrizzi, S. C., Sydora, B. C., Mikayama, T., Brossay, L. (2003) Differential regulation of killer cell lectin-like receptor G1 expression on T cells. *J. Immunol.* **170,** 5876–5885.
139. Thimme, R., Appay, V., Koschella, M., Panther, E., Roth, E., Hislop, A. D., Rickinson, A. B., Rowland-Jones, S. L., Blum, H. E., Pircher, H.

(2005) Increased expression of the NK cell receptor KLRG1 by virus-specific CD8 T cells during persistent antigen stimulation. *J. Virol.* **79,** 12112–12116.
140. Zajac, A. J., Vance, R. E., Held, W., Sourdive, D. J. D., Altman, J. D., Raulet, D. H., Ahmed, R. (1999) Impaired anti-viral T cell responses due to expression of the LY49A inhibitory receptor. *J. Immunol.* **163,** 5526–5534.
141. Phillips, J. H., Gumperz, J., Parham, P., Lanier, L. (1995) Superantigen-dependent, cell-mediated cytotoxicity inhibited by MHC class I receptors on T lymphocytes. *Science* **268,** 403–405.
142. Koh, C. Y., Blazar, B. R., George, T., Welniak, L. A., Capitini, C. M., Raziuddin, A., Murphy, W. J., Bennett, M. (2001) Augmentation of antitumor effects by NK cell inhibitory receptor blockade in vitro and in vivo. *Blood* **97,** 3132–3137.
143. Tajima, K., Matsumoto, N., Ohmori, K., Wada, H., Ito, M., Suzuki, K., Yamamoto, K. (2004) Augmentation of NK cell-mediated cytotoxicity to tumor cells by inhibitory NK cell receptor blockers. *Int. Immunol.* **16,** 385–393.
144. Wherry, E. J., Ha, S-J., Kaech, S. M., Haining, W. N., Sarkar, S., Kalia, V., Subramaniam, S., Blattman, J. N., Barber, D. L., Ahmed, R. (2007) Molecular signature of CD8+ T cell exhaustion during chronic viral infection. *Immunity* **27,** 670–684.
145. Goulder, P. J. R., Watkins, D. I. (2004) HIV and SIV CTL escape: implications for vaccine design. *Nat. Rev. Immunol.* **4,** 630–640.
146. Gulzar, N., Copeland, K. (2004) CD8+ T-cells: function and response to HIV infection. *Curr. HIV Res.* **2,** 23–37.
147. Yang, O. O. (2004) CTL ontogeny and viral escape: implications for HIV-1 vaccine design. *Trends Immunol.* **25,** 138–142.
148. De Maria, A., Moretta, L. (2000) HLA-class I-specific inhibitory receptors in HIV-1 infection. *Hum. Immunol.* **61,** 74–81.
149. Borg, C., Jalil, A., Laderach, D., Maruyama, K., Wakasugi, H., Charrier, S., Ryffel, B., Cambi, A., Figdor, C., Vainchenker, W., Galy, A., Caignard, A., Zitvogel, L. (2004) NK cell activation by dendritic cells (DCs) requires the formation of a synapse leading to IL-12 polarization in DCs. *Blood* **104,** 3267–3275.
150. Poggi, A., Carosio, R., Spaggiari, G. M., Fortis, C., Tambussi, G., Dell'Antonio, G., Dal Cin, E., Rubartelli, A., Zocchi, M. R. (2002) NK cell activation by dendritic cells is dependent on LFA-1-mediated induction of calcium-calmodulin kinase II: inhibition by HIV-1 Tat C-terminal domain. *J. Immunol.* **168,** 95–101.
151. Ferlazzo, G., Morandi, B., D'Agostino, A., Meazza, R., Melioli, G., Moretta, A., Moretta, L. (2003) The interaction between NK cells and dendritic cells in bacterial infections results in rapid induction of NK cell activation and in the lysis of uninfected dendritic cells. *Eur. J. Immunol.* **33,** 306–313.
152. Gerosa, F., Baldani-Guerra, B., Nisii, C., Marchesini, V., Carra, G., Trinchieri, G. (2002) Reciprocal activating interaction between natural killer cells and dendritic cells. *J. Exp. Med.* **195,** 327–333.
153. Zitvogel, L. (2002) Dendritic and natural killer cells cooperate in the control/switch of innate immunity. *J. Exp. Med.* **195,** F9–F14.
154. Semino, C., Angelini, G., Poggi, A., Rubartelli, A. (2005) NK/iDC interaction results in IL-18 secretion by DCs at the synaptic cleft followed by NK cell activation and release of the DC maturation factor HMGB1. *Blood* **106,** 609–616.
155. Semino, C., Ceccarelli, J., Lotti, L. V., Torrisi, M. R., Angelini, G., Rubartelli, A. (2007) The maturation potential of NK cell clones toward autologous dendritic cells correlates with HMGB1 secretion. *J. Leukoc. Biol.* **81,** 92–99.
156. Vitale, M., Chiesa, M. D., Carlomagno, S., Pende, D., Arico, M., Moretta, L., Moretta, A. (2005) NK-dependent DC maturation is mediated by TNF{α} and IFN{γ} released upon engagement of the NKp30 triggering receptor. *Blood* **106,** 566–571.
157. Xu, J., Chakrabarti, A. K., Tan, J. L., Ge, L., Gambotto, A., Vujanovic, N. L. (2007) Essential role of the TNF-TNFR2 cognate interaction in mouse dendritic cell-natural killer cell crosstalk. *Blood* **109,** 3333–3341.
158. Ferlazzo, G., Tsang, M. L., Moretta, L., Melioli, G., Steinman, R. M., Munz, C. (2002) Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKp30 receptor by activated NK cells. *J. Exp. Med.* **195,** 343–351.
159. Moretta, L., Ferlazzo, G., Bottino, C., Vitale, M., Pende, D., Mingari, M. C., Moretta, A. (2006) Effector and regulatory events during natural killer-dendritic cell interactions. *Immunol. Rev.* **214,** 219–228.
160. Terme, M., Tomasello, E., Maruyama, K., Crepineau, F., Chaput, N., Flament, C., Marolleau, J-P., Angevin, E., Wagner, E. F., Salomon, B., Lemonnier, F. A., Wakasugi, H., Colonna, M., Vivier, E,. Zitvogel, L. (2004) IL-4 confers NK stimulatory capacity to murine dendritic cells: a signaling pathway involving KARAP/DAP12-triggering receptor expressed on myeloid cell 2 molecules. *J. Immunol.* **172,** 5957–5966.
161. Munz, C., Steinman, R. M., Fujii, S-i. (2005) Dendritic cell maturation by innate lymphocytes: coordinated stimulation of innate and adaptive immunity. *J. Exp. Med.* **202,** 203–207.
162. Pende, D., Castriconi, R., Romagnani, P., Spaggiari, G. M., Marcenaro, S., Dondero, A., Lazzeri, E., Lasagni, L., Martini, S., Rivera, P., Capobianco, A., Moretta, L., Moretta, A., Bottino, C. (2006) Expression of the DNAM-1 ligands, Nectin-2 (CD112) and poliovirus receptor (CD155), on dendritic cells: relevance for natural killer-dendritic cell interaction. *Blood* **107,** 2030–2036.
163. Yang, T., Flint, M., Webb, K., Chambers, W. (2006) CD161B:ClrB interactions mediate activation of enhanced lysis of tumor target cells following NK cell:DC co-culture. *Immunol. Res.* **36,** 43–50.
164. Jinushi, M., Takehara, T., Kanto, T., Tatsumi, T., Groh, V., Spies, T., Miyagi, T., Suzuki, T., Sasaki, Y., Hayashi, N. (2003) Critical role of MHC class I-related chain A and B expression on IFN-{α}-stimulated dendritic cells in NK cell activation: impairment in chronic hepatitis C virus infection. *J. Immunol.* **170,** 1249–1256.
165. Jinushi, M., Takehara, T., Tatsumi, T., Kanto, T., Miyagi, T., Suzuki, T., Kanazawa, Y., Hiramatsu, N., Hayashi, N. (2004) Negative regulation of NK cell activities by inhibitory receptor CD94/NKG2A leads to altered NK cell-induced modulation of dendritic cell functions in chronic hepatitis C virus infection. *J. Immunol.* **173,** 6072–6081.
166. Pyzik, M., Piccirillo, C. A. (2007) TGF-{β}1 modulates Foxp3 expression and regulatory activity in distinct CD4+ T cell subsets. *J. Leukoc. Biol.* **82,** 335–346.
167. Mavilio, D., Lombardo, G., Kinter, A., Fogli, M., La Sala, A., Ortolano, S., Farschi, A., Follmann, D., Gregg, R., Kovacs, C., Marcenaro, E., Pende, D., Moretta, A., Fauci, A. S. (2006) Characterization of the defective interaction between a subset of natural killer cells and dendritic cells in HIV-1 infection. *J. Exp. Med.* **203,** 2339–2350.
168. Tasca, S., Tambussi, G., Nozza, S., Capiluppi, B., Zocchi, M., Soldini, L., Veglia, F., Poli, G., Lazzarin, A., Fortis, C. (2003) Escape of monocyte-derived dendritic cells of HIV-1 infected individuals from natural killer cell-mediated lysis. *AIDS* **17,** 2291–2298.
169. Quaranta, M. G., Napolitano, A., Sanchez, M., Giordani, L., Mattioli, B., Viora, M. (2007) HIV-1 Nef impairs the dynamic of DC/NK crosstalk: different outcome of CD56dim and CD56bright NK cell subsets. *FASEB J.* **21,** 2323–2334.
170. Mailliard, R. B., Son, Y-I., Redlinger, R., Coates, P. T., Giermasz, A., Morel, P. A., Storkus, W. J., Kalinski, P. (2003) Dendritic cells mediate NK cell help for Th1 and CTL responses: two-signal requirement for the induction of NK cell helper function. *J. Immunol.* **171,** 2366–2373.
171. Gerosa, F., Gobbi, A., Zorzi, P., Burg, S., Briere, F., Carra, G., Trinchieri, G. (2005) The reciprocal interaction of NK cells with plasmacytoid or myeloid dendritic cells profoundly affects innate resistance functions. *J. Immunol.* **174,** 727–734.
172. Funeshima, N., Fujino, M., Kitazawa, Y., Hara, Y., Hayakawa, K., Okuyama, T., Kimura, H., Li, X. (2005) Inhibition of allogeneic T-cell responses by dendritic cells expressing transduced indoleamine 2,3-dioxygenase. *J. Gene Med.* **7,** 565–575.
173. Munn, D. H., Sharma, M. D., Lee, J. R., Jhaver, K. G., Johnson, T. S., Keskin, D. B., Marshall, B., Chandler, P., Antonia, S. J., Burgess, R., Slingluff Jr., C. L., Mellor, A. L. (2002) Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. *Science* **297,** 1867–1870.
174. Fallarino, F., Grohmann, U., Vacca, C., Bianchi, R., Orabona, C., Spreca, A., Fioretti, M., Puccetti, P. (2002) T cell apoptosis by tryptophan catabolism. *Cell Death Differ.* **9,** 1069–1077.
175. Fuchs, D., Möller, A., Reibnegger, G., Stöckle, E., Werner, E., Wachter, H. (1990) Decreased serum tryptophan in patients with HIV-1 infection correlates with increased serum neopterin and with neurologic/psychiatric symptoms. *J. Acquir. Immune Defic. Syndr.* **3,** 873–876.
176. Roetynck, S., Baratin, M., Johansson, S., Lemmers, C., Vivier, E., Ugolini, S. (2006) Natural killer cells and malaria. *Immunol. Rev.* **214,** 251–263.
177. Chougnet, C. A., Shearer, G. (2007) Regulatory T cells (Treg) and HIV/AIDS: summary of the September 7–8, 2006 workshop. *AIDS Res. Hum. Retroviruses* **23,** 945–952.
178. Ghiringhelli, F., Menard, C., Martin, F., Zitvogel, L. (2006) The role of regulatory T cells in the control of natural killer cells: relevance during tumor progression. *Immunol. Rev.* **214,** 229–238.
179. Ahmad, A., Sharif-Askari, E., Fawaz, L., Menezes, J. (2000) Innate immune response of the human host to exposure with herpes simplex virus type 1: in vitro control of the virus infection by enhanced natural killer activity via interleukin-15 induction. *J. Virol.* **74,** 7196–7203.

180. Nguyen, K. B., Salazar-Mather, T. P., Dalod, M. Y., Van Deusen, J. B., Wei, X-q., Liew, F. Y., Caligiuri, M. A., Durbin, J. E., Biron, C. A. (2002) Coordinated and distinct roles for IFN-{α}{β}, IL-12, and IL-15 regulation of NK cell responses to viral infection. *J. Immunol.* **169,** 4279–4287.
181. Borghi, P., Fantuzzi, L., Varano, B., Gessani, S., Puddu, P., Conti, L., Capobianchi, M., Ameglio, F., Belardelli, F. (1995) Induction of interleukin-10 by human immunodeficiency virus type 1 and its gp120 protein in human monocytes/macrophages. *J. Virol.* **69,** 1284–1287.
182. Ito, M., Ishida, T., He, L., Tanabe, F., Rongge, Y., Miyakawa, Y., Terunuma, H. (1998) HIV type 1 Tat protein inhibits interleukin 12 production by human peripheral blood mononuclear cells. *AIDS Res. Hum. Retroviruses* **14,** 845–849.
183. Poggi, A., Zocchi, M. (2006) HIV-1 Tat triggers TGF-β production and NK cell apoptosis that is prevented by pertussis toxin B. *Clin. Dev. Immunol.* **13,** 369–372.
184. Pugliese, A., Vidotto, V., Beltramo, T., Torre, D. (2005) Regulation of interleukin-18 by THP-1 monocytoid cells stimulated with HIV-1 and Nef viral protein. *Eur. Cytokine Netw.* **16,** 186–190.
185. Quaranta, M. G., Camponeschi, B., Straface, E., Malorni, W., Viora, M. (1999) Induction of interleukin-15 production by HIV-1 Nef protein: a role in the proliferation of uninfected cells. *Exp. Cell Res.* **250,** 112–121.
186. Ahmad, R., Sindhu, S. T. A., Toma, E., Morisset, R., Ahmad, A. (2003) Studies on the production of IL-15 in HIV-infected/AIDS patients. *J. Clin. Immunol.* **23,** 81–90.
187. Chehimi, J., Starr, S., Frank, I., D'Andrea, A., Ma, X., MacGregor, R., Sennelier, J., Trinchieri, G. (1994) Impaired interleukin 12 production in human immunodeficiency virus-infected patients. *J. Exp. Med.* **179,** 1361–1366.
188. Fan, J., Bass, H. Z., Fahey, J. L. (1993) Elevated IFN-γ and decreased IL-2 gene expression are associated with HIV infection. *J. Immunol.* **151,** 5031–5040.
189. Saez, R., Echaniz, P., Juan, M. D. D., Iribarren, J. A., Cuadrado, E. (2007) The impaired response of NK cells from HIV-infected progressor patients to A-class CpG oligodeoxynucleotides is largely dependent of a decreased production of IL-12. *Immunol. Lett.* **109,** 83–90.
190. Feldman, S., Stein, D., Amrute, S., Denny, T., Garcia, Z., Kloser, P., Sun, Y., Megjugorac, N., Fitzgerald-Bocarsly, P. (2001) Decreased interferon-[α] production in HIV-infected patients correlates with numerical and functional deficiencies in circulating type 2 dendritic cell precursors. *Clin. Immunol.* **101,** 201–210.
191. Hosmalin, A., Lebon, P. (2006) Type I interferon production in HIV-infected patients. *J. Leukoc. Biol.* **80,** 984–993.
192. Lotz, M., Seth, P. (1993) TGF β and HIV infection. *Ann. N. Y. Acad. Sci.* **685,** 501–511.
193. Shearer, G. M., Clerici, M. (1998) Cytokine profiles in HIV type 1 disease and protection. *AIDS Res. Hum. Retroviruses* **14** (Suppl. 2), S149–S152.
194. Ahmad, R., Sindhu, S. T. A., Toma, E., Morisset, R., Ahmad, A. (2002) Elevated levels of circulating interleukin-18 in human immunodeficiency virus-infected individuals: role of peripheral blood mononuclear cells and implications for AIDS pathogenesis. *J. Virol.* **76,** 12448–12456.
195. Torre, D., Speranza, F., Martegani, R., Pugliese, A., Castelli, F., Basilico, C., Biondi, G. (2000) Circulating levels of IL-18 in adult and paediatric patients with HIV-1 infection. *AIDS* **14,** 2211–2212.
196. Ahmad, R., Iannello, A., Samarani, S., Morisset, R., Toma, E., Grosley, M., Ahmad, A. (2006) Contribution of platelet activation to plasma IL-18 concentrations in HIV-infected AIDS patients. *AIDS* **20,** 1907–1909.
197. MacPherson, P. A., Fex, C., Sanchez-Dardon, J., Hawley-Foss, N., Angel, J. (2001) Interleukin-7 receptor expression on CD8(+) T cells is reduced in HIV infection and partially restored with effective antiretroviral therapy. *J. Acquir. Immune Defic. Syndr.* **28,** 454–457.
198. Faller, E. M., McVey, M., Kakal, J., MacPherson, P. (2006) Interleukin-7 receptor expression on CD8 T-cells is downregulated by the HIV Tat protein. *J. Acquir. Immune Defic. Syndr.* **43,** 257–269.
199. Biron, C. A., Nguyen, K. B., Pien, G. C., Cousens, L. P., Salazar-Mather, T. P. (1999) Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu. Rev. Immunol.* **17,** 189–220.
200. Cooper, M. A., Fehniger, T. A., Turner, S. C., Chen, K. S., Ghaheri, B. A., Ghayur, T., Carson, W. E., Caligiuri, M. A. (2001) Human natural killer cells: a unique innate immunoregulatory role for the CD56bright subset. *Blood* **97,** 3146–3151.
201. Hoshino, T., Winkler-Pickett, R. T., Mason, A. T., Ortaldo, J. R., Young, H. A. (1999) IL-13 production by NK cells: IL-13-producing NK and T cells are present in vivo in the absence of IFN-{γ}. *J. Immunol.* **162,** 51–59.
202. Mehrotra, P. T., Donnelly, R. P., Wong, S., Kanegane, H., Geremew, A., Mostowski, H. S., Furuke, K., Siegel, J. P., Bloom, E. T. (1998) Production of IL-10 by human natural killer cells stimulated with IL-2 and/or IL-12. *J. Immunol.* **160,** 2637–2644.
203. Warren, H. S., Kinnear, B., Phillips, J., Lanier, L. (1995) Production of IL-5 by human NK cells and regulation of IL-5 secretion by IL-4, IL-10, and IL-12. *J. Immunol.* **154,** 5144–5152.
204. Furuke, K., Burd, P. R., Horvath-Arcidiacono, J. A., Hori, K., Mostowski, H., Bloom, E. T. (1999) Human NK cells express endothelial nitric oxide synthase, and nitric oxide protects them from activation-induced cell death by regulating expression of TNF-{α}. *J. Immunol.* **163,** 1473–1480.
205. Loza, M. J., Zamai, L., Azzoni, L., Rosati, E., Perussia, B. (2002) Expression of type 1 (interferon γ) and type 2 (interleukin-13, interleukin-5) cytokines at distinct stages of natural killer cell differentiation from progenitor cells. *Blood* **99,** 1273–1281.
206. Peritt, D., Robertson, S., Gri, G., Showe, L., Aste-Amezaga, M., Trinchieri, G. (1998) Cutting edge: differentiation of human NK cells into NK1 and NK2 subsets. *J. Immunol.* **161,** 5821–5824.
207. Takahashi, K., Miyake, S., Kondo, T., Terao, K., Hatakenaka, M., Hashimoto, S., Yamamura, T. (2001) Natural killer type 2 bias in remission of multiple sclerosis. *J. Clin. Invest.* **107,** R23–R29.
208. Borzychowski, A. M., Croy, B. A., Chan, W. L., Redman, C. W., Sargent, I. L. (2005) Changes in systemic type-1 and type-2 immunity in normal pregnancy and pre-eclampsia may be mediated by natural killer cells. *Eur. J. Immunol.* **35,** 3054–3063.
209. Wei, H., Zhang, J., Xiao, W., Feng, J., Sun, R., Tian, Z. (2005) Involvement of human natural killer cells in asthma pathogenesis: natural killer 2 cells in type 2 cytokine predominance. *J. Allergy Clin. Immunol.* **115,** 841–847.
210. Chan, W. L., Pejnovic, N., Lee, C. A., Al-Ali, N. A. (2001) Human IL-18 receptor and ST2L are stable and selective markers for the respective type 1 and type 2 circulating lymphocytes. *J. Immunol.* **167,** 1238–1244.
211. Carter, R. W., Sweet, M., Xu, D., Klemenz, R., Liew, F., Chan, W. (2001) Regulation of ST2L expression on T helper (Th) type 2 cells. *Eur. J. Immunol.* **31,** 2979–2985.
212. Barker, E., Mackewicz, C., Levy, J. (1995) Effects of TH1 and TH2 cytokines on CD8+ cell response against human immunodeficiency virus: implications for long-term survival. *Proc. Natl. Acad. Sci. USA* **92,** 11135–11139.
213. Romagnani, S. (1997) The Th1/Th2 paradigm. *Immunol. Today* **18,** 263–266.
214. Chehimi, J., Bandyopadhyay, S., Prakash, K., Perussia, B., Hassan, N. F., Kawashima, H., Campbell, D., Kornbluth, J., Starr, S. E. (1991) In vitro infection of natural killer cells with different human immunodeficiency virus type 1 isolates. *J. Virol.* **65,** 1812–1822.
215. Toth, F. D., Mosborg-Petersen, P., Kiss, J., Aboagye-Mathiesen, G., Zdravkovic, M., Hager, H., Ebbesen, P. (1993) Differential replication of human immunodeficiency virus type 1 in CD8– and CD8+ subsets of natural killer cells: relationship to cytokine production pattern. *J. Virol.* **67,** 5879–5888.
216. Vuillier, F., Bianco, N., Montagnier, L., Dighiero, G. (1988) Selective depletion of low-density CD8+, CD16+ lymphocytes during HIV infection. *AIDS Res. Hum. Retroviruses* **4,** 121–129.
217. Lusso, P., Malnati, M. S., Garzino-Demo, A., Crowley, R. W., Long, E. O., Gallo, R. C. (1993) Infection of natural killer cells by human herpesvirus 6. *Nature* **362,** 458–462.
218. Saha, K., Zhang, J., Gupta, A., Dave, R., Yimen, M., Zerhouni, B. (2001) Isolation of primary HIV-1 that target CD8+ T lymphocytes using CD8 as a receptor. *Nat. Med.* **7,** 65–72.
219. Valentin, A., Rosati, M., Patenaude, D. J., Hatzakis, A., Kostrikis, L. G., Lazanas, M., Wyvill, K. M., Yarchoan, R., Pavlakis, G. N. (2002) Persistent HIV-1 infection of natural killer cells in patients receiving highly active antiretroviral therapy. *Proc. Natl. Acad. Sci. USA* **99,** 7015–7020.
220. Harada, H., Goto, Y., Ohno, T., Suzu, S., Okada, S. (2007) Proliferative activation up-regulates expression of CD4 and HIV-1 co-receptors on NK cells and induces their infection with HIV-1. *Eur. J. Immunol.* **37,** 2148–2155.
221. Valentin, A., Pavlakis, G. (2003) Natural killer cells are persistently infected and resistant to direct killing by HIV-1. *Anticancer Res.* **23,** 2071–2075.
222. Chaudhary, P. M., Mechetner, E. B., Roninson, I. B. (1992) Expression and activity of the multidrug resistance P-glycoprotein in human peripheral blood lymphocytes. *Blood* **80,** 2735–2739.
223. Sastry, K. J., Marin, M., Nehete, P., McConnell, K., el-Naggar, A., McDonnell, T. (1996) Expression of human immunodeficiency virus type

I tat results in down-regulation of bcl-2 and induction of apoptosis in hematopoietic cells. *Oncogene* **13,** 487–493.

224. Kottilil, S., Shin, K., Jackson, J. O., Reitano, K. N., O'Shea, M. A., Yang, J., Hallahan, C. W., Lempicki, R., Arthos, J., Fauci, A. S. (2006) Innate immune dysfunction in HIV infection: effect of HIV envelope-NK cell interactions. *J. Immunol.* **176,** 1107–1114.
225. Espert, L., Denizot, M., Grimaldi, M., Robert-Hebmann, V., Gay, B., Varbanov, M., Codogno, P., Biard-Piechaczyk, M. (2006) Autophagy is involved in T cell death after binding of HIV-1 envelope proteins to CXCR4. *J. Clin. Invest.* **116,** 2161–2172.
226. Naora, H., Gougeon, M. (1999) Enhanced survival and potent expansion of the natural killer cell population of HIV-infected individuals by exogenous interleukin-15. *Immunol. Lett.* **68,** 359–367.
227. Huntington, N. D., Puthalakath, H., Gunn, P., Naik, E., Michalak, E. M., Smyth, M. J., Tabarias, H., Degli-Esposti, M. A., Dewson, G., Willis, S. N., Motoyama, N., Huang, D. C. S., Nutt, S. L., Tarlinton, D. M., Strasser, A. (2007) Interleukin 15-mediated survival of natural killer cells is determined by interactions among Bim, Noxa and Mcl-1. *Nat. Immunol.* **8,** 856–863.
228. Kim, H., Rafiuddin-Shah, M., Tu, H-C., Jeffers, J. R., Zambetti, G. P., Hsieh, J. J. D., Cheng, E. H. Y. (2006) Hierarchical regulation of mitochondrion-dependent apoptosis by BCL-2 subfamilies. *Nat. Cell Biol.* **8,** 1348–1358.
229. Ahmad, A., Ahmad, R., Iannello, A., Toma, E., Morisset, R., Sindhu, S. (2005) IL-15 and HIV infection: lessons for immunotherapy and vaccination. *Curr. HIV Res.* **3,** 261–270.
230. Jewett, A., Bonavida, B. (1996) Target-induced inactivation and cell death by apoptosis in a subset of human NK cells. *J. Immunol.* **156,** 907–915.
231. Jewett, A., Cavalcanti, M., Giorgi, J., Bonavida, B. (1997) Concomitant killing in vitro of both gp120-coated CD4+ peripheral T lymphocytes and natural killer cells in the antibody-dependent cellular cytotoxicity (ADCC) system. *J. Immunol.* **158,** 5492–5500.
232. Ida, H., Anderson, P. (1998) Activation-induced NK cell death triggered by CD2 stimulation. *Eur. J. Immunol.* **28,** 1292–1300.
233. Ortaldo, J. R., Mason, A., O'Shea, J. (1995) Receptor-induced death in human natural killer cells: involvement of CD16. *J. Exp. Med.* **181,** 339–404.
234. Yamauchi, A., Taga, K., Mostowski, H., Bloom, E. (1996) Target cell-induced apoptosis of interleukin-2-activated human natural killer cells: roles of cell surface molecules and intracellular events. *Blood* **87,** 5127–5135.
235. Ross, M. E., Caligiuri, M. A. (1997) Cytokine-induced apoptosis of human natural killer cells identifies a novel mechanism to regulate the innate immune response. *Blood* **89,** 910–918.
236. Shibatomi, K., Ida, K., Yamasaki, S., Nakashima, T., Origuchi, T., Kawakami, A., Migita, K., Kawabe, Y., Tsujihata, M., Anderson, P., Eguchi, K. (2001) A novel role for interleukin-18 in human natural killer cell death: high serum levels and low natural killer cell numbers in patients with systemic autoimmune diseases. *Arthritis Rheum.* **44,** 884–892.
237. Yang, Y., Dong, B., Mittelstadt, P. R., Xiao, H., Ashwell, J. D. (2002) HIV Tat binds Egr proteins and enhances Egr-dependent transactivation of the Fas ligand promoter. *J. Biol. Chem.* **277,** 19482–19487.
238. Dianzani, U., Bensi, T., Savarino, A., Sametti, S., Indelicato, M., Mesturini, R., Chiocchetti, A. (2003) Role of FAS in HIV infection. *Curr. HIV Res.* **1,** 405–417.
239. Loubeau, M., Ahmad, A., Toma, E., Menezes, J. (1997) Enhancement of natural killer and antibody-dependent cytolytic activities of the peripheral blood mononuclear cells of HIV-infected patients by recombinant IL-15. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol* **16,** 137–145.
240. Tanneau, F., McChesney, M., Lopez, O., Sansonetti, P., Montagnier, L., Rivière, Y. (1990) Primary cytotoxicity against the envelope glycoprotein of human immunodeficiency virus-1: evidence for antibody-dependent cellular cytotoxicity in vivo. *J. Infect. Dis.* **162,** 837–843.
241. Amiel, C., Béné, M., May, T., Canton, P., Faure, G. (1988) LFA1 expression in HIV infection. *AIDS* **2,** 211–214.
242. Zocchi, M. R., Rubartelli, A., Morgavi, P., Poggi, A. (1998) HIV-1 Tat inhibits human natural killer cell function by blocking L-type calcium channels. *J. Immunol.* **161,** 2938–2943.
243. Mazerolles, F., Barbat, C., Trucy, M., Kolanus, W., Fischer, A. (2002) Molecular events associated with CD4-mediated down-regulation of LFA-1-dependent adhesion. *J. Biol. Chem.* **277,** 1276–1283.
244. Nair, M. P. N., Schwartz, S. A. (1995) Synergistic effect of cortisol and HIV-1 envelope peptide on the NK activities of normal lymphocytes. *Brain Behav. Immun.* **9,** 20–30.
245. Peruzzi, M., Azzari, C., Rossi, M., De Martino, M., Vierucci, A. (2000) Inhibition of natural killer cell cytotoxicity and interferon γ production by the envelope protein of HIV and prevention by vasoactive intestinal peptide. *AIDS Res. Hum. Retroviruses* **16,** 1067–1073.
246. Lin, S. J., Roberts, R., Ank, B., Nguyen, Q., Thomas, E., Stiehm, E. (1998) Effect of interleukin (IL)-12 and IL-15 on activated natural killer (ANK) and antibody-dependent cellular cytotoxicity (ADCC) in HIV infection. *J. Clin. Immunol.* **18,** 335–345.
247. Mela, C. M., Burton, C., Imami, N., Nelson, M., Steel, A., Gazzard, B., Gotch, F., Goodier, M. (2005) Switch from inhibitory to activating NKG2 receptor expression in HIV-1 infection: lack of reversion with highly active antiretroviral therapy. *AIDS* **19,** 1761–1769.
248. Chehimi, J., Azzoni, L., Farabaugh, M., Creer, S. A., Tomescu, C., Hancock, A., Mackiewicz, A., D'Alessandro, L., Ghanekar, S., Foulkes, A. S., Mounzer, K., Kostman, J., Monaner, L. J. (2007) Baseline viral load and immune activation determine the extent of reconstitution of innate immune effectors in HIV-1-infected subjects undergoing antiretroviral treatment. *J. Immunol.* **179,** 2642–2650.
249. Koenig, S., Conley, A., Brewah, Y., Jones, G., Leath, S., Boots, L., Davey, V., Pantaleo, G., Demarest, J., Carter, C., et al. (1995) Transfer of HIV-1-specific cytotoxic T lymphocytes to an AIDS patient leads to selection for mutant HIV variants and subsequent disease progression. *Nat. Med.* **1,** 330–336.
250. Lieberman, J., Skolnik, P. R., Parkerson III, G. R., Fabry, J. A., Landry, B., Bethel, J., Kagan, J. (1997) Safety of autologous, ex vivo-expanded human immunodeficiency virus (HIV)-specific cytotoxic T-lymphocyte infusion in HIV-infected patients. *Blood* **90,** 2196–2206.
251. Iannello, A., Débbeche, O., Samarani, S., Sabbagh, S., Duval, M., Ahmad, A. (2007) Potential role of NK cells in future anti-tumor immunotherapies. *Med. Sci. (Paris)* **23,** 502–508.
252. Ruggeri, L., Aversa, F., Martelli, M. F., Velardi, A. (2006) Allogeneic hematopoietic transplantation and natural killer cell recognition of missing self. *Immunol. Rev.* **214,** 202–218.
253. Johnson, R. P., Trocha, A., Buchanan, T. M., Walker, B. D. (1993) Recognition of a highly conserved region of human immunodeficiency virus type 1 gp120 by an HLA-Cw4-restricted cytotoxic T-lymphocyte clone. *J. Virol.* **67,** 438–445.
254. Littaua, R. A., Oldstone, M. B., Takeda, A., Debouck, C., Wong, J. T., Tuazon, C. U., Moss, B., Kievits, F., Ennis, F. A. (1991) An HLA-C-restricted CD8+ cytotoxic T-lymphocyte clone recognizes a highly conserved epitope on human immunodeficiency virus type 1 gag. *J. Virol.* **65,** 4051–4056.
255. Wilson, C. C., Kalams, S., Wilkes, B., Ruhl, D., Gao, F., Hahn, B., Hanson, I., Luzuriaga, K., Wolinsky, S., Koup, R., Buchbinder, S., Johnson, R., Walker, B. (1997) Overlapping epitopes in human immunodeficiency virus type 1 gp120 presented by HLA A, B, and C molecules: effects of viral variation on cytotoxic T-lymphocyte recognition. *J. Virol.* **71,** 1256–1264.
256. Nehete, P. M., Lewis, D., Tang, D., Pollack, M., Sastry, K. (1998) Presence of HLA-C-restricted cytotoxic T-lymphocyte responses in long-term nonprogressors infected with human immunodeficiency virus. *Viral Immunol.* **11,** 119–129.
257. Pietra, G., Romagnani, C., Mazzarino, P., Falco, M., Millo, E., Moretta, A., Moretta, L., Mingari, M. C. (2003) HLA-E-restricted recognition of cytomegalovirus-derived peptides by human CD8+ cytolytic T lymphocytes. *Proc. Natl. Acad. Sci. USA* **100,** 10896–10901.
258. Altfeld, M., Kalife, E. T., Qi, Y., Streeck, H., Lichterfeld, M., Johnston, M. N., Burgett, N., Swartz, M. E., Yang, A., Alter, G., Yu, X. G., Meier, A., Rockstroh, J. K., Allen, T. M., Jessen, H., Rosenberg, E. S., Carrington, M., Walker, B. D. (2006) HLA alleles associated with delayed progression to AIDS contribute strongly to the initial CD8+ T cell response against HIV-1. *PLoS Med.* **3,** e403.
259. McMichael, A. J., Rowland-Jones, S. L. (2001) Cellular immune responses to HIV. *Nature* **410,** 980–987.
260. McCutcheon, J. A., Gumperz, J., Smith, K., Lutz, C., Parham, P. (1995) Low HLA-C expression at cell surfaces correlates with increased turnover of heavy chain mRNA. *J. Exp. Med.* **181,** 2085–2095.
261. Tong, J. C., Zhang, Z. H., August, J. T., Brusic, V., Tan, T. W., Ranganathan, S. (2007) In silico characterization of immunogenic epitopes presented by HLA-Cw*0401. *Immunome Res.* **3,** 7.
262. Albertini, M. R., Sosman, J., Hank, J., Moore, K., Borchert, A., Schell, K., Kohler, P., Bechhofer, R., Storer, B., Sondel, P. (1990) The influence of autologous lymphokine-activated killer cell infusions on the toxicity and antitumor effect of repetitive cycles of interleukin-2. *Cancer* **66,** 2457–2464.
263. Clark, J. W., Smith II, J. W., Steis, R. G., Urba, W. J., Crum, E., Miller, R., McKnight, J., Beman, J., Stevenson, H. C., Creekmore, S., Stewart, M., Conlon, K., Sznol, M., Kremers, P., Cohen, P., Longo, D. L. (1990) Interleukin 2 and lymphokine-activated killer cell therapy: analysis of a

bolus interleukin 2 and a continuous infusion interleukin 2 regimen. *Cancer Res.* **50,** 7343–7350.

264. Chen, W., Syldath, U., Bellmann, K., Burkart, V., Kolb, H. (1999) Human 60-kDa heat-shock protein: a danger signal to the innate immune system. *J. Immunol.* **162,** 3212–3219.
265. Kim, D-K., Kabat, J., Borrego, F., Sanni, T. B., You, C-h., Coligan, J. E. (2004) Human NKG2F is expressed and can associate with DAP12. *Mol. Immunol.* **41,** 53–62.
266. Tanaka, F., Hashimoto, W., Okamura, H., Robbins, P. D., Lotze, M. T., Tahara, H. (2000) Rapid generation of potent and tumor-specific cytotoxic T lymphocytes by interleukin 18 using dendritic cells and natural killer cells. *Cancer Res.* **60,** 4838–4844.
267. Carnaud, C., Lee, D., Donnars, O., Park, S-H., Beavis, A., Koezuka, Y., Bendelac, A. (1999) Cutting edge: cross-talk between cells of the innate immune system: NKT cells rapidly activate NK cells. *J. Immunol.* **163,** 4647–4650.
268. Gonzalez-Aseguinolaza, G., Van Kaer, L., Bergmann, C. C., Wilson, J. M., Schmieg, J., Kronenberg, M., Nakayama, T., Taniguchi, M., Koezuka, Y., Tsuji, M. (2002) Natural killer T cell ligand {α}-galactosylceramide enhances protective immunity induced by malaria vaccines. *J. Exp. Med.* **195,** 617–624.
269. Zhou, H., Luo, Y., Lo, J-f., Kaplan, C. D., Mizutani, M., Mizutani, N., Lee, J-D., Primus, F. J., Becker, J. C., Xiang, R., Reisfeld, R. A. (2005) DNA-based vaccines activate innate and adaptive antitumor immunity by engaging the NKG2D receptor. *Proc. Natl. Acad. Sci. USA* **102,** 10846–10851.
270. Tran, A. C., Zhang, D., Byrn, R., Roberts, M. R. (1995) Chimeric ζ-receptors direct human natural killer (NK) effector function to permit killing of NK-resistant tumor cells and HIV-infected T lymphocytes. *J. Immunol.* **155,** 1000–1009.
271. Gupta, N., Arthos, J., Khazanie, P., Steenbeke, T. D., Censoplano, N. M., Chung, E. A., Cruz, C. C., Chaikin, M. A., Daucher, M., Kottilil, S., Mavilio, D., Schuck, P., Sun, P. D., Rabin, R. L., Radaev, S., Van Ryk, D., Cicala, C,. Fauci, A. S. (2005) Targeted lysis of HIV-infected cells by natural killer cells armed and triggered by a recombinant immunoglobulin fusion protein: implications for immunotherapy. *Virology* **332,** 491–497.
272. Woll, P. S., Martin, C. H., Miller, J. S., Kaufman, D. S. (2005) Human embryonic stem cell-derived NK cells acquire functional receptors and cytolytic activity. *J. Immunol.* **175,** 5095–5103.

# 6- Mise à jour de la littérature concernant les cellules NK et l'infection par le VIH-1

Depuis la publication des deux articles précédents portant sur l'interation entre les cellules Natural Killer et l'infection par le VIH-1, plusieurs articles originaux contenant des informations intéréssantes et pertinentes sur ce sujet ont été publiés. Le chapitre suivant contient une synthèse des informations publiées depuis la parution de nos articles.

## 6-1 Interaction entre les cellules NK et les autres immunocytes

Des études récentes ont été menées afin de comprendre l'interaction entre les cellules dendritiques et les cellules NK lors de l'infection par le VIH-1 et le SIDA. L'étude menée par Saïdi et al. a démontré que l'interaction (*cross-talk*) entre les cellules dendritiques infectées par le VIH-1 et des cellules NK autologues était défective [239]. Cette interaction défectueuse résulte sur l'incapacité des CD à médier une polarisation des cellules T $CD4^+$ naïves vers un type Th1. Ces cellules dendritiques sont incapable de produire de l'IL-12 et de l'IL-18 et une réplication virale importante a été observée à l'intérieur de ces cellules. Il semble que la protéine HMGB1 (*High-mobility group box 1*), qui est produite par les deux types cellulaires, joue un rôle dans ce défaut car l'inhibition de cette dernière par la glycyrrhizine ou des anticorps neutralisants anti-HMGB1 a réduit la réplication virale. Cette étude représente l'une des première étude mettant en cause l'interaction entre ces deux cellules au niveau de la dissémination du virus. Une étude menée par la même équipe a démontré que les cellules dendritiques infectées par le VIH-1 pouvait échapper à la lyse médiée par les cellules NK et leur récepteur TRAIL (*TNF-Related Apoptosis-Induced Ligand*) [240]. Cet échappement est du à une augmentation de l'expression des protéines anti-apoptotiques c-FLIP (*cellular-Flice Inhibitory Protein*) et c-IAP2 (*cellular Inhibitor of Apoptosis 2*) par la protéine HMGB1.

Un défaut dans l'interaction entre les cellules NK et les cellules dendritiques plasmacytoïde (CDp) a été observé chez les patients infectés [241]. Les CDp des patients infectés ont une incapacité à produire de l'IFN-α et du TNF-α qui en contre-partie ne peuvent pas activer les cellules NK. Il semble aussi que les cellules NK de ces patients ont un défaut de réponse aux cytokines produites par les CDp, et que le défaut d'interaction provenait des deux types cellulaires et non seulement des CDp [242]. Cette étude a de plus démontré, comme il a été observé *in vivo*, que la gp120 (trimérique) du VIH-1 inhibe l'activité des cellules NK en se liant à l'intégrine α4β7 [241]. De plus, une étude a démontré que la protéine virale Vpr inhibe la production d'IFN de type I par les CDp sans induire leur apoptose. Cette inhibition aurait pour conséquence l'inhibiton de la production d'IFN-γ par les cellules NK lors de co-cultures autologues [243].

Une étude a démontré que, malgré l'expression de niveaux élevés du récepteur TRAIL à la surface des CDp de patients infectés, ces cellules sont dans l'incapacité de lyser les cellules T $CD4^+$ autologues qui expriment pourtant le récepteur DR5 (*Death Receptor 5*) [244]. Ceci documente donc un autre disfonctionnement de l'interaction entre ces deux types cellulaires lors de l'infection.

Une étude menée par Scott-Algara et al. a démontré que l'interaction entre des cellules dendritiques dérivées de monocytes infectées par le VIH-1 et des cellules NK autologues réduit l'expression de la protéine CD85j (aussi appelé *leukocyte Ig-like receptor-1/ILT2* ou LIR-1/ILT2) sur ces dernières [245]. Ils ont démontré de plus que les cellules NK $CD85j^+$ contrôlent mieux la réplication du VIH-1 lors de co-cultures *in vitro* que les cellules NK $CD85j^-$. Cette inhibition aurait lieu sans sécrétion de facteurs solubles et sans utiliser de cytotoxicité mais par liaison à un ligand inconnu, distinct des molécules du CMH-I, qui serait exprimé de façon préférentielle sur les cellules dendritiques infectées.

Le traitement de CD immatures avec de l'IL-10 induit des changements phénotypiques important. Ces CD deviennent résistantes à la lyse par les cellules NK et les CD matures deviennent très sensibles à une élimination par les cellules NK d'une manière NKG2D-dépendante [246]. L'infection des CD matures et immatures avec le VIH-1 induit leur sécrétion d'IL-10 ainsi que les mêmes résistances et susceptibilités que lors du traitement avec l'IL-10. De plus, les ganglions lymphatiques des patients infectés ont

une accumulation de CD partiellement immatures, ce qui corrobore les résultats obtenus *in vitro* par cette équipe [246]. Il semble donc que l'IL-10 perturbe le contrôle et l'homéostasie des CD médiés par les cellules NK au niveaux des organes lymphoïdes secondaires, ce qui aboutira au final à une mauvaise réponse adaptative.

### 6-2 Régulation des récepteurs et des ligands

Des études ont démontré que lors de l'infection par le VIH-1, les cellules T $CD4^+$ sur-exprimaient un ligand pour le récepteur activateur NKp44 (NKp44L) qui est exprimé par les cellules NK. L'expression de ce ligand rendrait ces cellules plus sensibles à la lyse médiée par les cellules NK. Les données démontrent que l'expression de ce ligand est déclenchée par un motif hautement conservé de la gp41 du VIH-1 (appelé 3S) [247]. Une de leurs études a démontré que lors de l'infection de macaques avec une souche CCR5 tropique SHIV, l'expression du NKp44L était inversement corrélée avec la quantié d'anticorps anti-3S. L'expression de ce ligand était aussi en relation avec la chute des cellules T $CD4^+$ et la cytotoxicité des cellules NK. Cependant, ces relations n'ont pas été observé lors de l'infection de macaques avec une souche double tropique [248]. Ceci suggère que l'utilisation de CCR5 ou de CXCR4 lors de l'infection influence la relation entre la déplétion des cellules T $CD4^+$, l'expression du NKp44L et la cytotoxicité des cellules NK [249].

Chez les patients infectés par le VIH-1, la quantité d'anticorps anti-3S était en relation avec la maintenance des cellules T $CD4^+$. Cette équipe a donc chercher à déterminer si l'immunisation avec un anti-3S pouvait prévenir l'expression du NKp44L *in vivo* afin d'inhiber la déplétion des cellules T $CD4^+$. Leur étude montre que les anticorps anti-3S inhibe l'expression du NKp44L et inhibent la susceptibilité des cellules T $CD4^+$ à la lyse par les cellules NK. De plus, ces anticorps réduisent le taux d'apoptose des cellules T $CD4^+$ au niveau du sang périphérique et des noeuds lymphatiques [248]. Cette équipe a de plus démontré que la protéine virale Nef présente au niveau des cellules T $CD4^+$ infectées et produisant du virus, pouvait inhiber l'expression en surface du NKp44L, rendant ces cellules non susceptibles à la lyse par les cellules NK. Cette étude a permis

de mettre à jour un autre des nombreux mécanismes d'évasions médié par la protéine virale Nef [250].

Récemment, deux études distinctes ont démontré que lorsque la protéine virale Vpr active la voie de stress et de dommage à l'ADN médiée par la voie ATR/ATM (kinases), ce qui permet l'arrêt du cycle cellulaire dans la phase G2. Cette protéine virale induit aussi l'expression de l'ULBP-1, ULBP-2 (*UL-16 Binding Protein-1 and -2*) et MIC-A et B (*MHC class I Chain-related gene A and B*) sur des cellules T CD4$^+$. Cette expression augmente de plus leur susceptibilité à la lyse médiée par les cellules NK [251, 252].

Le mécanisme d'ADCC médié par les cellules NK est directement lié à l'expression des récepteurs de la partie Fc des anticorps comme le récepteur FcγRIIIa (CD16a). L'expression de ce récepteur est lié à la présence et l'activité des métalloprotéases de matrice (MMP). L'étude menée par Liu et al. a démontré que l'inhibition de ces MMP résultait sur une augmentation de l'expression de CD16 et augmentait de ce fait l'ADCC médiée par les cellules NK [253]. Ces données suggèrent une nouvelle stratégie afin d'augmenter la réponse ADCC antivirale médiée par les cellules NK.

Une étude récente menée par Brunetta et al. a montré que seulement les patients co-infectés par le VIH-1 et le HCMV avaient une expansion des cellules NK exprimant le récepteur NKG2C [254]. Sachant que l'expression du récepteur NKG2A est réduite chez les patients chroniquement infectés, le ratio entre NKG2A et NKG2C a été inversé uniquement au niveau des cellules NK de patients infectés par ces deux virus. Une normalisation de ce ratio a été observée seulement 24 mois après la suppression de la réplication virale grâce à la thérapie. Ainsi, l'inversion du ratio NKG2A/NKG2C caractérise un stage avancé de la pathologie associée au patients co-infectés, rendant ce groupe de patients distinct des LTNP et des patients en primo-infection. Une autre étude réalisée par la même équipe a démontré une réduction drastique d'un récepteur particulier à la surface des cellules NK appelés Siglec-7 (*Sialic Acid-Binding Immunoglobulin-Like Lectin-7*) [255]. Il semble que la sous-régulation de ce récepteur ait lieu avant celle du CD56 lors de l'établissement de l'infection chronique. Ceci permet donc de différencier deux sous-types de cellules NK qui sont Siglec-7$^-$/CD56$^+$ et Siglec-7$^-$/CD56$^-$ lors de l'infection par le VIH-1, le premier sous-type apparaissant avant le second. De plus, l'apparition de ces sous-types est en relation avec un début de

disfonctionnement observable chez des patients qui ont un haut taux de réplication du VIH-1. Les patients non virémiques ou ayant un faible virémie ainsi que les patients sous thérapie antivirale n'ont d'aileurs pas d'apparition de ces sous-types. Ceci suggère donc l'utilisation de ces deux sous-types comme marqueurs de l'activité antivirale des cellules NK [255].

Récemment, une étude menée par Leeansyah et al. a démontré que les niveaux de FcRγ au niveau des cellules NK $CD56^{+}CD94^{+}$ étaient réduits chez les patients infectés sous traitement antirétroviral [256].

### 6-3 Perturbation de l'homéostasie des sous-types de cellules NK

L'infection par le VIH-1 entraîne une perte des cellules NK $CD56^{dim}CD16^{+}$. Une étude récente a démontré que parmis ces cellules, seules les cellules étant $CD57^{-}$ ou $CD57^{dim}$ et non les $CD57^{bright}$ sont déplétées lors de l'infection chronique. Il semble que l'augmentation de l'expression de CD57 soit associée à une haute expression des KIR et de granzyme B ainsi qu'une réduction de CD107a, Ki-67, HLA-DR et CD27. Il semble donc que l'infection par le VIH-1 induit une déplétion des cellules NK qui sont moins différenciées [257]. Une étude menée par Reeves et al. a montré que chez les macaques infectés par le VIS, les cellules NK circulantes $CD16^{+}$ ainsi que les $CD56^{-}CD16^{-}$ sont augmentées en nombre et bien que leurs marqueurs de cytotoxicité augmentent, leur sécrétion de cytokine réduit [258]. Leurs cellules NK $CD56^{+}$ ont des niveaux élevés anormaux de perforine, granzyme B et CD107a. Au contraire, les molécules permettant le *homing* comme le CD62L et CCR7, qui sont normalement exprimées sur les cellules $CD56^{+}$, sont sous-régulées lors de l'infection. Ces données démontrent donc que l'infection par le VIS entraîne un *shift* dans les fonctions des cellules NK qui est caractérisé par une baisse dans la production des cytokines, de la cytotoxicité, et du trafic à partir des organes lymphoïdes secondaires [258].

### 6-4 Les cellules Natural Killer utérines

Une étude a démontré que les cellules NK provenant de mères et d'enfants pouvaient répondre avec spécificité à une stimulation par des peptides appartenant au VIH-1 (enveloppe et régions régulatrices). Ceci permettrait que l'enfant ne soit pas infecté malgré une exposition au virus *in utero* [259]. De façon similaire, l'étude menée par Mselle et al. a démontré que seules les cellules NK utérines et non les cellules NK du sang périphérique sont capables de produire plusieurs facteurs solubles, notamment la chimiokine CXCL12, et que ces cellules sont capable d'inhiber la réplication du VIH-1 [260]. Ces facteurs pourraient donc contribuer à l'inhibition de la transmission du virus à travers les tissus et les muqueuses lors de la grossesse.

### 6-5 Études épistatiques sur les KIR et le CMH-I

L'expression combinée du récepteur KIR3DS1 et son ligand HLA-Bw4-80I a été associée lors d'études épidémiologiques à une lente progression du SIDA. Il semble que le fait d'avoir une homozygotie pour le gène KIR3DS1 permette à des enfant exposés au VIH-1 de ne pas être infectés [261].

L'étude menée par Alter et al. a démontré *in vitro* que les cellules NK exprimant ce récepteur pouvaient mieux inhiber la réplication du VIH-1 d'une façon dose-dépendante par contact avec des cellules cibles exprimant le HLA-Bw4-80I, par rapport aux cellules qui n'expriment pas le récepteur KIR3DS1 [262]. De plus, ces cellules NK lysent les cellules cibles infectées d'une manière dépendante du HLA-Bw4-80I. Il semble de plus que les cellules NK exprimant le récepteur KIR3DS1, et à un moindre niveau KIR3DL1, prolifèrent lors de l'infection aigüe par le VIH-1 en présence de cellules exprimant le HLA-Bw4-80I [263].

Une étude menée par la même équipe démontre que l'expression des KIR sur les cellules T $CD8^+$ est progressivement augmentée lors de l'infection par le VIH-1 et corrèle avec le niveau de réplication virale. L'expression des KIR est associée avec une inhibition drastique de la sécrétion de cytokines, de la dégranulation, de la prolifération et de

l'activation de ces cellules après une stimulation de leurs TCR. La suppression des fonctions des cellules T CD8$^{+}$ KIR$^{+}$ est indépendante de l'engagement de leurs ligands, ce qui suggère que ces récepteurs répriment de façon constitutive l'activation de leur TCR [264].

Les personnes qui expriment une seule copie du gène KIR3DS1 ont des cellules NK qui produisent plus d'IFN-γ et de CD107a au niveau de leurs cellules NK au repos ainsi que lors de l'activation de ces cellules. Cependant, il semble que l'augmentation de ces fonctions effectrices due à la présence du gène KIR3DS1 ne soit pas obligatoirement liée à la présence du HLA-Bw4-80I chez ces patients [265].

Une étude réalisée *in vivo* sur des macaques rhésus infectés par le VIS a démontré qu'un haut niveau de réplication virale et une baisse dans la fréquence et la fonction des cellules NK étaient associés avec une augmentation des niveaux d'ARNm codant pour la protéine KIR3DL1. De plus, le polymorphisme observable au niveau du génomedes différents macaques était en relation avec le taux de réplication du VIS *in vivo*. Il semblerait que la présence d'un SNP 159H/Q au niveau de ce gène soit en relation avec une virémie élevée [266].

Cependant, l'étude menée par O'Connell et al. démontre que seulement certain patients de leur cohorte de contrôleurs élites possèdent des cellules NK capables d'inhiber la réplication du VIH-1 *in vitro* [267]. Il semble que leur cohorte n'exprime pas de façon plus élevé les récepteurs KIR3DS1 ainsi que les autres récepteurs KIR. De plus, les cellules NK de patients permettant la meilleure inhibition *in vitro* étaient celles ayant le moins d'expression des récepteurs KIR activateurs et possédaient un haplotype A. Ces données suggèrent donc qu'au niveau des contrôleurs élites, l'inhibition de la réplication virale ne soit pas médié par les cellules NK exprimant les récepteurs KIR activateurs.

Une étude a démontré une reconnaissance de peptides appartenant à la gp120 du VIH-1 (SF9 et son variant SL9, respectivement SFNCGGEFF et SFNCGGEFL) par les cellules T cytotoxiques ainsi que les cellules NK qui expriment le récepteur inhibiteur KIR2DL1 [268]. En effet, l'utilisation d'un tétramère HLA-Cw4 chargé avec ces peptides a démontré que SF9 était reconnu par les les cellules T cytotoxiques et que SL9 était mieux reconnu que SF9 par les cellules NK. La liaison de SF9 sur le récepteur KIR2DL1 a résulté sur l'inhibition des fonctions de ces cellules. Ainsi, ces données

démontrent que la variation au niveau de ce peptide de la gp120 correspondant au peptide SL9 permet d'échapper à la reconnaissance par les cellules T cytotoxiques en inhibant en parallèle l'activité des cellules NK, un phénomène que ces scientifiques ont appelé "double échappement".

Une étude récente utilisant une cohorte Sud-Africaine contenant des personnes adultes infectées et sans traitement a démontré que les patients virémiques ayant atteints un stade avancé de la maladie n'exprimaient pas le récepteur NKp46 à la surface de leurs cellules NK [269]. De plus, les fréquences des cellules NK exprimant les récepteurs KIR2DS1/KIR2DL1 étaient réduites chez ces patients.

## 7- Dérégulation dans la production de cytokines lors de l'infection par le VIH-1 et le développement du SIDA

La prolifération, la différenciation ainsi que les fonctions des cellules du système immunitaire sont contrôlées en partie par les cytokines, et particulièrement celles partageant la chaîne commune $\gamma$ au niveau de leur récepteur [71, 270].

Lors de l'infection par le VIH-1 et le développement du SIDA, les défauts observés dans les fonctions, la maintenance, ainsi que la consistance des cellules du système immunitaire sont en large partie attribués à une production altérée des cytokines et/ou à un manque d'efficacité au niveau de leurs effets biologiques.

En effet, la compréhension des mécanismes moléculaires responsables des perturbations du réseau cytokinique pourrait aider à fournir des nouvelles stratégies thérapeutiques pour le traitements des patients infectés afin de restaurer leur réponse immunitaire antivirale. De tels efforts sont nécessaires puisque le traitement anti-rétroviral ne permet pas d'éliminer totalement le VIH-1 du corps, possède des effets secondaires non négligeables, et ne restaure que partiellement le système immunitaire de l'hôte.

### 7-1 L'interleukine-2 dans l'infection par le VIH-1

Il n'est toujours pas clair de nos jours si les niveaux sériques de l'IL-2 sont perturbés chez les patients infectés car plusieurs études se contredisent. La perturbation dans la production d'IL-2 ainsi que l'absence de son effet biologique chez les patients infectés par le VIH-1 ont été découvertent en 1984 [271]. Les premières études sur le sujet ont démontré que, bien avant la chute du taux de cellules T $CD4^+$, les patients infectés et assymptomatiques pouvaient être stratifiés en deux groupes basés sur la fonction de leurs cellules Th lors d'essais *in vitro*. Ces fonctions correspondent à la capacité de leurs cellules à sécréter de l'IL-2 et à proliférer lors d'une activation par différents antigènes de rappel (influenza et toxine tétanique), des allo-antigènes (activation par un mélange de lymphocytes), ainsi que des agents mitogènes comme la phytohaemagglutinine (PHA) [272, 273]. Le traitement de patients virémiques avec des inhibiteurs de transcriptase inverse a permis d'augmenter le niveau d'expression de l'IL-2R sur leurs cellules T $CD8^+$ par rapport aux patients non traités [274]. Cependant, la réponse à l'IL-2, mesurée par la prolifération de ces cellules, n'a pas été améliorée.

Les patients sous HAART ont aussi subi une augmentation du niveau d'expression de l'IL-2R sur leurs cellules T $CD8^+$. Ces cellules ont de plus acquis la capacité de répondre et de proliférer en présence d'IL-2. Le même comportement fut observé au niveau de leurs cellules T $CD4^+$, bien que l'expression de l'IL-2R observée fut plus faible que celle présente au niveau de cellules T $CD8^+$. Ceci suggère donc l'existance d'un blocage du signal médié par l'IL-2 chez les patients virémiques traités par des inhibiteurs de transcriptase inverse.

En effet, les études qui ont suivi ont permis de montrer que les cellules T $CD8^+$ de certains de ces patients virémiques non-traités étaient incapable d'activer STAT-5 en réponse à une stimulation par l'IL-2 [275]. Bien que la capacité de l'IL-2R à lier l'IL-2 n'était pas altérée, une activation altérée de la kinase Jak3 en amont de STAT-5 fut observée. Les patients traités sous HAART pendant une période de six mois ont subi une restauration partielle de l'activation de leur voie Jak/STAT induite par l'IL-2. La capacité de l'IL-2 à activer les voies des MAP kinases et de la PI-3 kinase au niveau de leurs cellules T reste encore à être étudiée.

### 7-2 L'interleukine-4 dans l'infection par le VIH-1

Clerici et Shearer ont proposé originalement un modèle dans lequel les patients infectés par le VIH-1 développant le SIDA subissent un changement dans la production de cytokines de type Th1 vers le type Th2 [273, 276, 277]. Ceci est basé sur l'idée qu'une réponse cytokinique de type Th1 devrait avoir un effet protecteur contre l'infection alors que la production de cytokines de type Th2 devrait contribuer à la progression du SIDA. Cependant, des données contradictoires ont remis en question cette hypothèse [278-280]. Certains groupes ont observé une augmentation de la production d'IL-4, alors que d'autres une réduction de cette dernière lors de l'infection par le VIH-1 [273, 276, 278-280]. Des études ont en effet démontré que les patients possédant un taux de cellules T $CD4^+$ < 400/µl ou >400/µl avaient respectivement une réduction et une augmentation de la sécrétion d'IL-4 lors d'une stimulation mitogénique *in vitro* [281]. Les études réalisées sur de multiples cohortes de patients séropositifs à des stades différents du SIDA ont suggéré que les patients considérés Long-Term Non-Progressors (LTNP, étant infectés et non-traités depuis plus de 8 ans, sans symptômes et avec un taux de cellules T $CD4^+$ normal) avaient un profil de production cytokinique de type Th1, alors que les patients progresseurs avaient un profil d'expression de type Th2 [281-284]. Une augmentation dans les niveaux d'IgE a aussi été observée par différents groupes chez les patients infectés [283, 285-288]. Becker et al. ont proposé que le modèle de switch Th1 vers Th2 dans la production de cytokines chez les patients souffrant d'allergies s'appliquait aussi pour les patients infectés par le VIH-1 [289, 290]. Ce changement dans la production de cytokines pourrait créer des conditions non favorables à l'éradication du virus en empêchant la production de cytokines de type Th1, en inhibant la réponse CTL, et en causant une augmentation de l'expression du co-récepteur CXCR4. Une augmentation de l'expression de CXCR4 pourrait augmenter la réplication du VIH-1 et la création de nouveaux variants [289, 291].

Au niveau du récepteur, quelques études ont identifié des SNP (*Single Nucleotide Polymorphism*) dans le gène codant pour le récepteur à l'IL4, et les ont associés à une progression du SIDA [292-294]. Il a été suggéré que certains de ces SNP, qui sont liés au manque de réponse après stimulation par l'IL-4, seraient associés à une baisse de la

progression de la maladie [293]. D'autres haplotypes communs dans l'exon 12 du récepteur, possiblement associés à une augmentation de la réponse médiée par l'IL-4, pourraient conférer une résistance à l'infection transmise de façon sexuelle alors qu'ils augmenteraient la transmission parentérale du virus [293]. Cependant d'autres études sont requises afin de confirmer ces observations. Aucune donnée à ce jour n'existe sur l'expression de l'IL-4R au niveau des cellules T concernant les différents groupes de patients infectés par le VIH-1. Cependant, des résultats récent suggèrent que les niveaux d'expression de l'IL-4R ne sont pas altérés chez les patients traités avec HAART, et les patients non traités par rapport aux contrôles. En effet, l'activation de STAT-6 en réponse à l'IL-4 n'est pas affectée tant au niveau des cellules T $CD4^+$ et $CD8^+$ chez ces patients [295].

### 7-3 L'interleukine-7 dans l'infection par le VIH-1

Les niveaux sériques de l'IL-7 augmentent lors de l'infection par le VIH-1. Napolitano et al. ont été les premiers à démontré cette augmentation et à l'associer à une haute charge virale à tous les stages de la maladie étudiés [296]. Ils ont de plus démontré que ces niveaux élevés étaient inversement corrélés avec les taux de cellules T $CD4^+$. Des études subséquentes ont confimé cette augmentation et ont montré que les niveaux d'IL-7 corrélaient avec la baisse du récepteur à l'IL-7 (CD127) sur les cellules T $CD8^+$ chez les patients infectés [297]. Ces niveaux élevés ont aussi été démontrés chez les patients au stade de la primo-infection [298, 299]. Une corrélation inverse entre les niveaux d'IL-7 plasmatiques et le taux de cellules T $CD4^+$ a été observée seulement chez les patients au stade de l'infection chronique. De plus, le traitement anti-rétroviral (HAART) permet de normaliser de façon significative les niveaux d'IL-7 chez les patients en primo-infection, bien que ces niveaux restent élevés chez les patients chroniquement infectés [299]. Récemment, Albuquerque et al. ont observé une augmentation dans les niveaux d'IL-7, bien que retardée, lors de la chute progressive des cellules T $CD4^+$ chez les patients infectés par le VIH-2 [300].

Une des premières hypothèse émise était celle que les niveaux élevés d'IL-7 étaient dus à la déplétion des cellules T exprimant l'IL-7R dans la circulation des patients inféctés, créant ainsi des conditions ne permettant pas l'absorption des niveaux élevés constants d'IL-7 par ces dernières [296]. Cependant, il est plus probable que les niveaux d'IL-7 soient augmentés à cause d'une réponse immune active, ceci étant due à un état de lymphopénie ressentie par les cellules stromales non lymphoïdes, qui sont les principales cellules productrices d'IL-7 [296].

Les analyses histochimiques suggérent que l'IL-7 serait produite par les macrophages et des cellules de type cellules dendritiques dans les noeuds lymphatiques périphériques. L'augmentation dans la production de l'IL-7 a principalement lieu dans les tissus lymphatiques des patients infectés par le VIH-1 qui sont depletés de leur lymphocytes [296]. Récemment, Fluur et al. ont montré que, contrairement aux patients non traités, les niveaux d'IL-7 sont significativement plus bas chez les LTNP [301]. De plus, les LTNP qui ont perdu leurs statut de non progresseur ont subi une augmentation concernant leurs niveaux d'IL-7 sériques. D'une manière générale, il semble donc que l'augmentation des niveaux d'IL-7 soit une réponse directe de l'organisme pour répondre et restaurer la quantité de cellules T pour éviter une lymphopénie. De ce fait, les patients qui sont capable de contrôler l'infection ne possède pas ce besoin et produisent donc des niveaux normaux d'IL-7 [301]. Cependant, ces niveaux élevés d'IL-7 peuvent avoir des effets secondaires néfastes comme l'augmentation du niveau d'expression de Fas et l'apoptose que ce récepteur peut induire au niveau des cellules T [302-304]. L'expression de Fas est augmentée au niveau des cellules $TCD4^+$ et $CD8^+$ de macaques auxquels on a administré de l'IL-7 [302]. De plus, les niveaux d'IL-7 plasmatiques de patients infectés par le VIH-1 corrèlent avec l'expression de Fas sur leurs cellules T naïves et mémoires ainsi qu'avec leur susceptibilité à l'apoptose induite par Fas *ex vivo*.

Il est de nos jours bien établi que les niveaux d'expression de l'IL-7R sur les cellules $TCD4^+$, et de façon plus évidente sur les cellules T $CD8^+$, sont réduits chez les patients infectés par le VIH-1 [297, 299, 300, 305-310]. Cette baisse semble apparaître lors de la primo-infection ainsi qu'au stade chronique de la maladie [299]. Le traitement anti-rétroviral (HAART) semble restaurer de façon partielle l'expression de ce récepteur

[305-307]. Certaines de ces études ont de plus démontré une corrélation inverse entre l'expression du récepteur et les quantités d'IL-7 plasmatiques, la charge virale, l'activation immune, et la susceptibilité à l'apoptose, alors qu'une corrélation positive a été trouvée avec les comptes de cellules T $CD4^+$ des patients. Il a de plus été suggéré que les populations de cellules T $CD8^+$ ayant une expression réduite de l'IL-7R correspondent à une expansion des cellules T effectrices/mémoires ($T_{EM}$, $CCR7^-$, $CD62L^-$, $CD45RA^{+/-}$) [307]. Contrairement aux cellules exprimant l'IL-7R, les cellules qui expriment faiblement ce récepteur produisent plus d'IFN-γ, moins d'IL-2, sont plus susceptible à la mort cellulaire induite par activation (AICD) et à une apoptose spontanée. Les cellules exprimant faiblement l'IL-7R prolifèrent peu *ex vivo* après une stimulation mitogénique. Elle semble déjà avoir subit une forte prolifération car elles expriment un haut niveau de Ki-67. Il a été suggéré que l'augmentation des cellules T $CD8^+IL\text{-}7R^-$ lors de l'infection serait due à une stimulation antigénique plutôt qu'aux taux élevés d'IL-7 plasmatiques. Ces cellules contribueraient, bien qu'elles en soient aussi le résultat, à l'activation chronique de la réponse immune durant l'infection [307]. Des études ont montré que les cellules T $CD8^+IL\text{-}7R^+$ de patients infectés expriment des niveaux faibles du facteur de transcription GFI (*Growth Factor Independence*) par rapport aux cellules T $CD8^+IL\text{-}7R^-$ [295]. Rethi et al. ont montré que les cellules T $CD3^+$ de type mémoire possèdent une faible expression d'IL-7R qui est aussi associée à une baisse d'expression du CD28 [297]. Boutboul et al. ont aussi démontré que l'expression de l'IL-7R sur les cellules T $CD8^+$ des patients infectés était inversement corrélée avec l'expression de perforine, et que les niveaux d'expression de l'IL-7R sur les différents sous-types des cellules T $CD8^+$ spécifiques au VIH-1, à l'EBV (*Epstein Barr Virus*) et au HCMV (*Human Cytomegalovirus*) étaient équivalent [308].

Chez les patients infectés par le VIH-2, l'expression réduite de l'IL-7R semble plus apparaître au niveau des sous-types de cellules T $CD4^+$ mémoires et ces niveaux semblent maintenus sur les cellules naïves [300]. Bien que le pourcentage de cellules positives pour l'IL-7R soit réduit dans tous leurs sous-types de cellules $TCD8^+$, les patients infectés par le VIH-2 possèdent une meilleure préservation de l'expression de ce récepteur dans toutes les populations de cellules $TCD8^+$ de type effectrices/mémoires par rapport aux patients infectés par le VIH-1. Ces différents effets ne semblent pas être

dus à une différence au niveau de l'état d'activation des cellules entre les différents type de patients. De plus, l'étude réalisée par Rethi et al. démontre que l'expression de l'IL-7R reste faible sur les cellules T des patients infectés lors de culture *ex vivo* en absence d'IL-7 [297]. Ceci suggère que la baisse d'expression de l'IL-7R sur les cellules T pourrait être due à un mécanisme complexe et serait multi-factorielle. Faller et al. ont d'ailleurs démontré que la protéine Tat du VIH-1 était capable de sous-régulé l'expression de l'IL-7R à la surface des cellules T CD8$^+$ primaires *in vitro* [311, 312].

Une baisse dans la capacité de réponse à l'IL-7 a de plus été observée chez les patients infectés par le VIH-1. Une étude cross-sectionelle sur les cellules T CD4$^+$ et CD8$^+$ de patients non traités et virémiques ainsi que sur des patients sous HAART démontre que les patients non traités ont une augmentation atténuée de l'expression de Bcl-2, CD25 et de l'activation de STAT-5 en réponse à l'IL-7 par rapport aux contrôles [295, 305]. Le traitement (HAART) a permis de restaurer de façon partielle l'augmentation de Bcl-2, l'activation de STAT-5 au niveau des deux types cellulaires, alors que l'expression de CD25 a été restaurée de façon significative seulement sur les cellules T CD4$^+$. La prolifération des PBMC en réponse à l'IL-7 était réduite chez les patients virémiques par rapport aux patients non infectés, et la prolifération était partiellement restaurée chez les patients traités. De plus, le traitement avec l'IL-7 de cellules T exprimant faiblement l'IL-7R a permis d'augmenter de façon réduite la survie de ces cellules par rapport au cellules de personnes non infectées [297]. Une autre étude a démontré que, la corrélation positive entre l'expression de l'IL-7R et l'augmentation IL-7-dépendante de Bcl-2 et CD25 dans les cellules T CD4$^+$ observable chez les patients non infectés, était perdue chez les patients virémiques et traités [313]. Ceci suggère que l'expression réduite de l'IL-7R n'est pas le seul phénomène entraînant une absence du rôle joué par l'IL-7 et que ce défaut de réponse peut persister chez les patients traités. Cependant, Vassena et al. ont démontré que l'IL-7 était tout de même toujours capable de réduire l'apoptose spontanée des cellules T de patients infectés lors de cultures *ex vivo*, et que cet effet de survie était inversement corrélé avec les taux de cellules T CD4$^+$ circulantes [314].

Une étude a évaluée l'activation des STAT par l'IL-7 dans des cellules T CD8$^+$ exprimant des niveaux faibles et élevés d'IL-7R chez des patients chroniquement infectés [295]. Leurs résultats démontrent que l'IL-7 n'arrive pas à activer STAT-5 dans

la majorité des cellules T $CD8^+$ de patients. Ce manque de stimulation corrèle avec la réduction de l'ARNm codant pour l'IL-7R et son expression en surface. Les cellules exprimant de faible niveaux d'IL-7 semblent être capable de recruter la réponse médiée par les STAT en réponse à l'IL-2, l'IL-4, l'IL-10 et l'IL-15. Il semble que le facteur de croissance indépendent GFI-1 réprime la transcription de l'IL-7R dans ces cellules [295].

Récemment, une étude menée par Crawley et al. a démontré que la forme soluble de l'IL-7R (CD127 soluble) inhibe la signalisation et l'activité biologique de l'IL-7 [315]. La prolifération cellulaire médiée par l'IL-7 ainsi que son activité anti-apoptotique ont donc été inhibées. Il semble que ce CD127 soluble soit présent dans les sérums de patients infectés par le VIH-1 et inhibe de façon plus importante l'activité de l'IL-7 que le CD127 soluble recombinant [315]. Ceci pourrait donc expliquer en partie le manque d'efficacité de l'IL-7 chez les patients infectés.

### 7-4 L'interleukine-10 dans l'infection par le VIH-1

L'interleukine-10 joue un rôle critique dans l'équilibre entre l'immunopathologie et la réponse immunitaire protectrice lors d'infection par différents pathogènes [316, 317]. L'IL-10 est une cytokine pléïotropique qui est produite par de multiples populations cellulaires et qui possède différents effets sur les cellules de types hématopoïétiques [317]. L'IL-10 peut jouer un rôle activateur aussi bien qu'immuno-suppresseur sur les cellules du système immunitaire [318]. La capacité de l'IL-10 à inhiber la production de cytokines par les cellules T ou les cellules NK semble être indirect en altérant la fonction des monocytes et des macrophages [319]. Ces études indiquent qu'un équilibre finement réglé entre les mécanismes pro-inflammatoires et l'effet immunosuppresseur médié par l'IL-10 est critique pour obtenir une éradication totale des agents pathogènes sans endommager l'hôte infecté.

Des niveaux élevés d'IL-10 (protéine et ARNm) ont été observés dans différentes maladies infectieuses chroniques chez l'homme comme la leishmaniose, la lèpre et la tuberculose [317]. Le fait même que l'EBV et le HCMV soient capable de produire des

protéines analogues à l'IL-10 suggère que l'IL-10 peut faciliter la persistence de ces virus chez l'homme [320, 321]. Lors de l'infection chronique par le VIH-1, la concentration d'IL-10 plasmatique augmente avec le temps. Une étude réalisée sur 51 patients infectés a démontré que les différents groupes de patients étudiés avaient des niveaux élevés d'IL-10 sériques par rapport aux personnes non infectées [322]. Les niveaux les plus élevés ont été observés chez les patients ayant atteint le stade du SIDA et particulièrement ceux souffrant d'infection complexe par *Mycobacterium avium*. Sur les 32 patients suivis longitudinalement, les patients progressant dans la maladie étaient ceux ayant des niveaux élevés d'IL-10 sériques par rapport aux patients qui ne progréssaient pas vers le stade SIDA, pour qui les niveaux d'IL-10 étaient stables. Bien que l'IL-10 et le TNF-$\alpha$ augmentent chez les patients progresseurs, le ratio IL-10/TNF-$\alpha$ était bas chez ces patients, ce qui suggère un déséquilibre entre ces deux cytokines [322]. Leurs études ont aussi démontré que les patients sous HAART subissent une baisse partielle mais significative des concentrations d'IL-10 sériques. Les recherches menées par Clerici et al. ont été les premières à démontrer que les PBMC de patients infectés qui sont stimulés par des mitogènes produisaient plus d'IL-10 que les PBMC de patients sains [323]. De plus, le fait de bloquer l'effet biologique de l'IL-10 par un anticorps neutralisant augmente la réponse proliférative des cellules T spécifiques à l'enveloppe du VIH-1. D'autre études démontrent que l'inhibition de l'IL-10 restaure la réponse proliférative des cellules T spécifiques à l'enveloppe du VIH-1 chez des patients ayant des taux de cellules T CD4$^+$ préservés, ce qui n'est pas le cas pour des patients qui sont à un stade avancé de l'infection [324]. De plus, leur niveau élevé d'IL-10 corrèle avec un stade avancé de la maladie. Ces données suggèrent donc un rôle pathogénique de l'IL-10 dans l'infection par le VIH-1.

La protéine virale Tat affecte le système immunitaire de l'hôte en influencant la production de cytokines comme l'IL-10. Une étude a démontré que Tat induit l'expression de l'IL-10 dans des monocytes de patients non infectés par le VIH-1 [325]. Leurs résultats démontrent que Tat induit un signal calcique qui régule l'expression de l'IL-10 dans ces cellules. La calmoduline, la protéine kinase-II dépendante de la calmoduline (CaMK-II) ainsi que la MAP kinase p38 vont augmenter la sécrétion d'IL-

10 dans les monocytes. Les protéines p38 et CaMK II vont permettre l'activation des facteurs de transcriptions CREB-1 et Sp-1, permettant ainsi l'expression de l'IL-10.

### 7-5 L'interleukine-12 dans l'infection par le VIH-1

L'interleukine-12 joue un rôle critique dans la réponse immune dirigée contre le VIH-1. Elle possède un rôle clef permettant la prolifération et la production d'IFN-γ par les cellules T et les cellules NK, influence la présentation antigénique, et régule la fonction des macrophages et des cellules dendritiques [326]. En régulant l'activité cytotoxique des cellules T et des cellules NK, l'IL-12 joue un rôle primordial dans la réponse antivirale, qui est reconnue pour être affaiblie et non fonctionnelle chez les patients infectés par le VIH-1. Les études sur l'expression de l'IL-12 chez ces patients sont assez contreversées. Une étude a démontré que les patients infectés possèdent des niveaux sériques élevés d'IL-12p40, d'IL-12p70 et d'IFN-γ par rapport aux patients non infectés [327]. Cependant, les niveaux d'IFN-γ et d'IL-12 ne semblent pas varier selon la progression vers l'immunodéficience. De plus, leur étude réalisée sur la sécrétion d'IL-12 par les PBMC de ces patients n'a pas mis en évidence un défaut dans l'expression d'IL-12.

Plusieurs autres études ont montré que les PBMC de patients infectés par le VIH-1 produisent faiblement de l'IL-12, notamment en réponse à l'infection par *Staphylococcus aureus Cowan* [328]. Lors d'une autre étude, des scientifiques ont stimulé des PBMC de patients infectés par le VIH-1 avec différents stimuli correspondant à des agents pathogènes avec ou sans traitement par de l'IFN-γ ou de l'IL-4, qui sont des cytokines reconnues pour activer la production d'IL-12. Les auteurs ont observé une réduction de l'ARNm codant pour les sous-unités p40 et p35 de l'IL-12 qui ont engendré une faible sécrétion d'IL-12 par les cellules de patients infectés [329]. Les PBMC de ces patients étaient aussi incapable d'augmenter l'expression de la chaîne β2 de l'IL-12R en réponse à une stimulation mitogénique. Cependant, l'expression de l'IL-12Rβ2 a été restaurée après traitement de ces cellules avec l'IL-12. Il est donc possible qu'un défaut primaire dans la production d'IL-12 conduise à une déficience dans

l'expression de l'IL-12Rβ2 et de l'IFN-γ, ce qui amplifie la déficience du système immunitaire chez les patients infectés. De plus, une étude a montré que le défaut dans la production d'IL-12 n'est pas due à l'hyperproduction d'IL-10, une cytokine reconnue pour agir de façon négative sur la production d'IL-12. Le défaut serait directement lié à une capacité réduite des monocytes à produire cette cytokine comme ceci a été observé lors d'infections *in vitro* [330].

Une étude a démontré que la production de l'IL-12p40 était réduite dans les noeuds lymphatiques de macaques infectés par le VIS par rapport aux macaques infectés avec un virus mutant délété pour la protéine virale Nef (VISmac239Δnef) [331]. Ceci suggère un rôle clef de cette protéine virale dans la régulation de l'IL-12 lors de l'infection. Récemment, une étude a démontré que l'expression intracellulaire de Nef dans des monocytes primaires ainsi que dans la lignée pro-monocytique THP-1 résulte sur l'inhibition de la production d'IL-12p40 lors d'une stimulation avec du LPS [332]. Il semble que Nef inhibe la transcription de l'IL-12 en inhibant l'activation des kinases JNK sans affecter le signal calcique lors d'une stimulation avec du LPS. Cette inhibition de JNK conduit à l'inhibition du facteur de transcription NFκB sans affecter l'activité d'AP-1.

Il semble de même que la gp120 et les protéines Tat et Vpr du VIH-1 inhibent la production de l'IL-12 au niveau des monocytes ou des PBMC totaux par des mécanismes directs ou indirects [333-335].

L'importance de cette cytokine dans la mise en place et la régulation de la réponse antivirale rend donc sa déficience comme l'une des raisons majeures de l'immunodéficience associée à l'infection par le VIH-1 et au développement du SIDA.

### 7-6 L'interleukine-15 dans l'infection par le VIH-1

Les niveaux sériques d'IL-15 ne semblent pas être affectés lors de la primo-infection [298]. Cependant, des résultats contradictoires concernant les concentrations sériques d'IL-15 ont été obtenus lors de l'étude de patients chroniquement infectés. Kacani et al. ont montré une augmentation significative des niveaux d'IL-15 par rapport

aux personnes non infectées et aucune différence n'a été observée entre les différemts groupes de patients infectés [336]. Cependant, lors de cette étude, les concentrations d'IL-15 sériques corrèlaient avec les niveaux d'immunoglobines sériques de type IgG. D'autres études ont démontré que les niveaux d'IL-15 sériques étaient diminués chez les patients infectés [337]. Les PBMC de ces patients avaient une faible activité de leurs cellules NK et une faible production d'IL-15 en réponse à l'infection par le *Herpes Simplex Virus-1* (HSV-1) [337, 338]. De plus, les patients avec des hauts taux de cellules T $CD4^+$ avaient une meilleure capacité à produire l'IL-15 lors d'une infection par le HSV-1 par rapport aux patients avec des faibles taux de cellules T $CD4^+$. Le même résultat a été observé lors de la stimulation de PBMC provenant de patients avec la souche A du *Staphylococcus aureus* [339]. Les résultats obtenus par d'Ettore et al. semblent être consistant à ces études. En effet, la stimulation de PBMC provenant de patients non traités ou de patients qui ne répondent pas au traitement avec du LPS et *Mycobacterium avium* n'a pas augmenté la production d'IL-15 [340]. Les PBMC de patients qui répondent au HAART ont quant à eux répondu à la stimulation en produisant de l'IL-15 à un niveau comparable à ceux des donneurs sains.

Il a été suggéré que les réponses spécifiques au VIH-1 médiées par les LTC sont fonctionnellement réduites et phénotypiquement immatures par rapport à celles dirigées contre le HCMV ou l'EBV chez les même patients infectés [341, 342]. Brenchley et al. ont fournit l'évidence d'une incapacité des LTC spécifiques au VIH-1 à proliférer lors d'une stimulation antigénique. Cette incapacité étant en corrélation inverse avec l'expression de CD57 [343]. De plus, cette incapacité de prolifération ne pouvait pas être restaurer par un traitement avec l'IL-2 ou l'IL-15. Cependant, il a été démontré que les LTC spécifiques au VIH-1 répondent à l'IL-15 *ex vivo* lors d'une culture prolongée *in vitro* [344]. L'IL-15 inhibe l'apoptose spontanée ainsi que celle médiée par Fas, augmente la survie des LTC spécifiques au VIH-1 en culture tout en induisant leur activation, augmente la production d'IFN-γ et leur activité cytotoxique. La stimulation par l'IL-15 augmente de manière générale les fonctions effectrices des LTC et confère une protection à l'apoptose médiée par Fas sur les cellules T $CD8^+$ de type effectrices/mémoires ainsi que les cellules T $CD4^+$ des patients infectés [345]. La capacité des monocytes à produire de l'IL-8 et la chimiokine MCP-1 en réponse à l'IL-

15 est affaiblie chez les patients non-traités et ceux ayant échoué au traitement (HAART) par rapport aux personnes non infectées et aux patients qui répondent au traitement [340].

L'ARNm codant pour l'IL-15R a été détécté à de faible niveaux dans les cellules T de poumon ainsi que dans les macrophages alvéolaires de patients infectés et symptomatiques [346]. Il a aussi été suggéré que l'expression de l'ARNm codant pour l'IL-15R est augmenté dans les PBMC de patients infectés par le VIH-1 et l'EBV lors de la phase aigüe de l'infection par rapport aux contrôles non infectés [347]. L'expression en surface de l'IL-15R est indétéctable au niveau des PBMC au repos de personnes non infectées mais le devient après stimulation par de la PHA [348]. De manière similaire, les cellules T périphériques ($CD4^+$ et $CD8^+$) expriment de faible voire d'indétéctable niveaux d'IL-15R bien que l'activation de STAT-5 (médiée par l'IL-15) par l'IL-2Rβ et la γc soit comparable entre les patients non traités, les patients sous HAART, et les personnes non infectées [295]. De plus, Pahwa et al. ont récemment démontré que l'expression de l'IL-15R était faible au niveau des sous-types de cellules T $CD8^+$ et que cette expression ne semble pas différer entre les personnes saines et les patients pédiatriques infectés par le VIH-1 [349]. La prolifération de ces cellules T $CD8^+$ en réponse à l'IL-15 ne sembe pas être affectée chez ces patients.

### 7-7 Le TGF-β dans l'infection par le VIH-1

Le *Transforming Growth Factor-beta* (TGF-β) est une cytokine pléïotropique ayant des effets multiples sur différentes cellules du système immunitaire. Plusieurs évidences suggèrent que cette cytokine est impliquée dans la pathogénèse lors de l'infection par le VIH-1. Des études ont permis de mettre en relation l'expression de cette cytokine et la progression de l'infection. Une étude réalisée sur 66 patients correspondant à différents stages de l'infection a démontré que les concentrations sériques du TGF-β étaient significativement augmentées chez les patients infectés par rapport aux donneurs sains [350]. Une corrélation négative a été observée entre les concentrations de TGF-β et les taux de cellules T $CD4^+$ et $CD8^+$. De plus, les patients se

situant dans la phase symptomatique de l'infection ont présenté des niveaux plasmatiques de TGF-β qui étaient deux fois plus élevés par rapport aux patients situés dans la phase asymptomatique ainsi que les patients non infectés. Plusieurs études démontrent aussi la relation entre les niveaux de TGF-β et l'avancement de l'infection, qui est d'autant plus observable dans les stades avancés de la maladie [350, 351].

Des études ont été menées sur le rôle du TGF-β dans la perte de fonction des cellules immunitaires chez les patients infectés par le VIH-1. Elles montrent que le TGF-β peut être détecté à la surface des cellules dendritiques de patients infectés par le VIH-1. Le fait de bloquer l'activité biologique du TGF-β situé à la membrane des cellules dendritiques lors d'une co-culture avec des cellules T autologues restaure les fonctions stimulatrices des cellules T nécessaires pour médier une réponse immunitaire efficace [351]. L'effet suppresseur de cette cytokine semble donc jouer un rôle clef dans la pathogénèse associée au VIH-1.

### 7-8 Le traitement anti-rétroviral (HAART) et la thérapie par les cytokines

Le traitement anti-rétroviral est très efficace pour augmenter les taux de cellules T $CD4^+$ et baisser la charge virale afin d'augmenter la survie des patients infectés par le VIH-1 [55]. Cependant, il est de nos jours reconnu que ce traitement n'est pas capable de supprimer de façon complète la réplication du VIH-1 ou d'éliminer totalement le virus présent dans l'organisme [352, 353]. De plus, il existe un véritable problème de résistance au traitement et la restauration du système immunitaire des patients traités reste partielle [354]. Chez certains de ces patients ayant reçu un traitement prolongé, le taux de cellules T $CD4^+$ n'arrive pas à augmenter malgré un contrôle de la réplication du virus, ce qui les rend plus susceptible aux infections opportunistes. Sachant que les cytokines de type $\gamma_c$ ont un effet positif sur la croissance, la différenciation et la survie des cellules T, leur utilisation thérapeutique en parallèle avec le traitement anti-rétroviral pourrait permettre une meilleure reconstitution immunitaire chez ces patients. Une étude récente menée par Stacey et al. a permis la caractérisation de la réponse immunitaire et spécialement la production de plusieurs facteurs solubles au niveau de la phase aigüe de

l'infection par le VIH-1 [355]. L'augmentation de la virémie a été associée à une augmentation transiente d'IFN-α et d'IL-15, une grande augmentation d'IP-10 (*Inhibitory Protein-10*) et une augmentation rapide et constante de TNF-α et de MCP-1 (*Monocyte Chemotactic Protein-1*). Une augmentation lente d'IL-6, d'IL-18, d'IL-18 et d'IFN-γ et une augmentation tardive des niveaux d'IL-10. Ces augmentations rapides et intenses dans la production de cytokines lors de la phase aigüe jouent donc sûrement un rôle dans l'activation de la réponse immunitaire et la réplication virale ainsi que sur l'immunopathologie associée au VIH-1. Ces données confirment donc une production non coordonnée des cytokines et chimiokines très tôt au cours de l'infection et relancent l'élaboration de stratégies thérapeutiques ciblant ces facteurs solubles lors de l'infection par le VIH-1.

La première cytokine à avoir été considérée dans ce type de traitement a été l'IL-2 [356]. Beaucoup d'études ont démontré son effet positif sur la restauration des taux de cellules T $CD4^+$ [357]. L'analyse de différentes études cliniques indépendantes démontre que l'IL-2 associée au HAART augmente le taux de ces cellules par rapport aux groupes de patients qui ont seulement reçu le HAART. De même, les patients qui ont reçu de l'IL-2 ont aussi subi une baisse significative de leur virémie [358]. Une autre étude démontre que l'IL-2 est aussi capable d'augmenter l'expression de Bcl-2 ainsi que la réponse immune mediée par cette cytokine. Cet effet est de plus corrélé avec une réduction dans la susceptibilité à l'apoptose [359-361]. Une étude clinique a démontré que les patients (avec un stade modéré de l'infection) sous HAART qui ont reçu de l'IL-2 possèdent une augmentation significative de cellules T $CD4^+$ naïves, mémoires et effectrices, ainsi que moins de symptômes classiques normalement présent chez les patients sidéens par rapport aux patients seulement sous HAART [362]. Les effets de l'IL-2 chez des patients sans HAART ont aussi été étudiés au niveau clinique. Les résultats démontrent que l'administration intermitente sous-cutanée d'IL-2 est bien tolérée et augmente significativement le taux de cellules T $CD4^+$ sans augmenter la charge virale des patients [363]. Cependant, l'utilisation clinique de l'IL-2 est associée à une toxicité, particulièrement lorsqu'elle est administrée à haute doses par voie intraveineuse Son utilisation conduit à des problèmes respiratoires aigües et de l'hypotension associée à des syndrômes de fuite vasculaire [364, 365]. Des données

récentes concernant le rôle critique de l'IL-2 dans le contrôle de la production des cellules Trég remet en question les implications cliniques de l'IL-2. L'utilisation de l'IL-2 peut augmenter le nombre de ces cellules Trég, entraînant ainsi une diminution des réponses effectrices antivirales. D'un autre point de vue, l'IL-2 pourrait permettre de balancer, via les Trég, les réponses médiées par les cellules T $CD4^+$ et $CD8^+$ pour prévenir de l'auto-immunité et restaurer un équilibre au niveau du système immunitaire antiviral. Le traitement des patients avec l'IL-2 résulte en fait sur une perturbation de l'homéostasie des cellules T $CD4^+$ malgré une augmentation du nombre de cellules T $CD4^+$ naïves et mémoires. Ceci pourrait être due à l'expansion clonale des cellules T $CD4^+/CD25^+$ (représentant des cellules Trég) observée dans ces conditions expérimentales [366-368]. Une étude intéréssante a démontré que la déplétion des cellules Trég à partir de PBMC de patients augmente la production de cytokines au niveau des cellules T $CD4^+$ et $CD8^+$ spécifiques au VIH-1 [369-371].

Du à son effet important sur l'homéostasie des cellules T, l'IL-7 a été considérée comme un candidat thérapeutique pour reconstituer le système immunitaire des patients et restaurer les taux de cellules T $CD4^+$ naïves et mémoires chez les patients infectés. Une étude clinique utilisant l'IL-7 chez des patients atteints de mélanomes métastatiques et de sarcomes métastatiques ont montré une expansion des cellules T $CD4^+$ et $CD8^+$ accompagnée par une baisse des cellules Trég [372]. Cette étude démontre aussi que l'IL-7 est bien tolérée chez tous les patients testés et n'est pas toxique pour l'organisme. Des études cliniques de phase I impliquant l'administration de l'IL-7 à des patients infectés par le VIH-1 sont en cours [373]. Les patients infectés et traités par HAART tolèrent l'IL-7 et exhibent une augmentation significative des taux de cellules T $CD4^+$ et $CD8^+$ sans aucune augmentation de leur charge virale après 52 semaines de traitement. Les récents essais cliniques ont montré que sur un temps court, l'administration de l'IL-7 résulte sur une expansion préférentielle des cellules T $CD4^+$ et $CD8^+$ naïves et mémoires avec une nette tendance dans l'augmentation des cellules T $CD8^+$. Ainsi, une expansion dans le *pool* de cellules T circulantes ayant un répertoire diversifié a été observée [374].

Les essais réalisés pour tester la toxicité de ce traitement montrent que le traitement est potentiellement réalisable. L'administration sous-cutanée d'une seule dose d'IL-7

recombinante (3 μg/kg) est bien tolérée et la dose maximum tolérée est de 30 μg/kg [375, 376]. Les effets secondaires remarquables ont été une réaction au site de l'administration ainsi qu'une activité hépatique élevée. Une augmentation transitoire de la virémie a été observée sur 6 des 11 patients traités. L'IL-7 a induit la prolifération des cellules T $CD4^+$ et $CD8^+$ et a conduit à une sous-régulation de l'IL-7R sur les deux types cellulaires. L'injection d'IL-7 a augmenté le nombre circulant de cellules T $CD4^+$ et $CD8^+$ de phénotype mémoire centrale. La fréquence des cellules T $CD4^+$ de phénotype $CD25^{high}$ $IL\text{-}7R^{low}$ n'a pas changé après l'injection d'IL-7 [377].
Lors d'une autre étude clinique de phase I/IIa [373], l'efficacité et la toxicité de l'IL-7 ont été évaluées sur 13 patients traités par traitement anti-rétroviral (non virémiques) ayant des taux de cellules T $CD4^+$ entre 100 et 400 cellules/μl. Les patients ont reçu 8 injections sous-cutanées de deux différentes doses d'IL-7 (3 ou 10 μg/kg) trois fois par jour sur une période de 16 jours. L'IL-7 semble avoir été bien tolérée chez ces patients en induisant une augmentation dans le nombre de cellules T $CD4^+$ et $CD8^+$ naïves et de phénotype mémoire centrale. Dans le groupe traité avec la haute dose d'IL-7, une augmentation transitoire de la réplication virale a été observée. Les essais fonctionnels ont démontré que les cellules T qui ont proliféré répondent à une stimulation antigénique spécifique au VIH-1 en produisant de l'IFN-γ et de l'IL-2. Chez les patients lymphopéniques, la thérapie avec l'IL-7 a induit des changements fonctionnels quantifiables au niveau de leurs cellules T pendant 48 semaines.

L'IL-15 semble aussi représenter un candidat potentiel prometteur pour ces patients en raison de ses effets importants sur la réponse immunitaire innée et acquise [378]. Plusieurs études encourageantes ont démontré que l'IL-15 est moins toxique que l'IL-2 au niveau du développement des syndrômes de fuite vasculaire et semble plus efficace que cette dernière quant à son rôle dans la réponse antivirale [379]. De même, l'administration *in vivo* d'IL-15 exogène a permis de protéger des souris contre une infection par HSV-2 reconnue pour être léthale normalement [380]. L'IL-15 a permis d'augmenter l'expansion des cellules NK et des cellules T $CD8^+$ après l'infection par ce virus. Jusqu'à présent, l'administration de l'IL-15 chez les patients infectés est évaluée en tant qu'adjuvant vaccinal dans différentes études.

## 8- Contexte de l'étude

Les patients séropositifs pour le VIH-1 vont développer de façon variable le SIDA. Bien que l'on comprenne de mieux en mieux le développement de la maladie et la pathogenèse associée à l'infection par le VIH-1, aucun traitement vraiment efficace n'est encore disponible. Les drogues modernes dirigées contre le VIH-1 ne permettent que de retarder la mortalité chez les patients et les projets permettant l'utilisation d'un vaccin anti-VIH ne sont malheureusement pas prometteurs.

Beaucoup de questions et de recherches ont été entreprises concernant la réponse immune antivirale adaptative chez les patients infectés par le VIH-1 et développant un SIDA, qu'elle soit de type cellulaire ou humoral. Cependant, moins d'attention a été portée à la compréhension de la réponse immunitaire innée contre cet agent pathogène. L'importance de l'immunité innée dans la protection de l'hôte ainsi que son rôle clé dans l'induction et le développement d'une immunité cellulaire adaptative dirigée de façon spécifique contre ce virus représente un domaine d'étude très faiblement exploré et qui est seulement en train d'émerger. Par conséquent, nous avons un besoin certain de mieux comprendre la réponse immune innée établie et développée chez les patients infectés par le VIH-1.

Il est bien reconnu de nos jours que lors de l'infection par le VIH-1, la production de cytokines ainsi que leur effets biologiques sur les différentes composantes du système immunitaire sont fortement déréglés (voir chapitre 7). Lors de ma thèse de doctorat, je me suis intéréssé pricipalement à deux cytokines qui sont l'interleukine-18 et l'interleukine-21.

Sachant que ces deux cytokines jouent un rôle majeur aussi bien dans la réponse antivirale permettant de contrôler l'infection, que dans l'immunopathogenèse associée à l'infection par le VIH-1 et le développement du SIDA, nous nous sommes intéréssés à la régulation et à l'effet biologique de ces deux facteurs chez les patients infectés par le VIH-1.

## 9- Hypothèses émisent lors de l'étude

Avant le début de mon doctorat, absolument aucune information n'était disponible concernant la régulation de l'IL-21 lors de l'infection par le VIH-1. Sachant que l'IL-21 est principalement produite par les lymphocytes T $CD4^+$ activés, qui sont aussi la cible du VIH-1, l'étude de cette cytokine nous semblait donc particulièrement relevante. Concernant l'interleukine-18, le laboratoire d'accueil dans lequel j'ai réalisé ces travaux avait auparavant travaillé sur cette cytokine et démontré que les concentrations sériques de cette dernière étaient anormalement élevés chez les patients infectés par le VIH-1. Sachant que ces deux cytokines jouent un rôle majeur dans la réponse antivirale et que leur production ainsi que leurs effets biologiques semblaient être perturbés, nous avons étudié ces deux cytokines de façon parallèle.

### 9-1 Hypothèses concernant l'interleukine-21

Nous émettons donc l'hypothèse que la production d'IL-21 est compromise chez les patients infectés par le VIH-1, ceci étant dû à l'infection même et à la déplétion quasi-totale des cellules T $CD4^+$. Cette déficience pourrait causer un défaut dans la maturation et la fonctionnalité des cellules effectrices du système immunitaire chez les patients infectés. De plus, nous émettons l'hypothèse que la présence d'IL-21 exogène pourrait restaurer les capacités fonctionnelles de ces cellules.

### 9-2 Hypothèses concernant l'interleukine-18

Danc cette partie de l'étude, nous avons émis l'hypothèse que les niveaux élevés d'interleukine-18 pouvaient avoir un effet considérable sur l'homéostasie et la fonctionnalité des cellules Natural Killer chez les patients infectés par le VIH-1. Nous avons de plus émis l'hypothèse qu'un déséquilibre entre l'IL-18 et son antagoniste naturel, l'*IL-18 binding protein* (IL-18BP) pouvait exister chez ces patients. Ce déséquilibre pourrait avoir des conséquences néfastes sur la réplication du VIH-1 et l'immunopathogenèse associée au SIDA.

## 10- Objectifs de l'étude

Concernant les travaux qui ont porté sur l'interleukine-18, les objectifs du projet de recherche étaient de savoir :

1. Comment l'IL-18 et son antagoniste naturel l'IL-18BP sont régulées chez les patients infectés par le VIH-1.

2. Quel est l'impact des niveaux élevés de l'IL-18 sur l'homéostasie et la fonctionnalité des cellules NK chez les patients infectés par le VIH-1.

3. Quel est l'impact de la dérégulation de cette cytokine (et de son antagoniste) sur la réplication du VIH-1 et sur l'immnunopathogénèse associée à l'infection par le VIH-1 et au SIDA.

Concernant les travaux qui ont porté sur l'interleukine-21, les objectifs du projet de recherche étaient de savoir :

1. Comment l'IL-21 est régulée chez les patients infectés par le VIH-1.

2. Comment l'infection par le VIH-1 affecte la production d'IL-21 *in vitro* et quels sont les mécanismes moléculaires entrant en jeu.

3. Comment l'IL-21 affecte la réplication du VIH-1 dans les cellules du système immunitaire humain.

4. Si l'IL-21 exogène pourrait restaurer les fonctions effectrices des cellules du système immunitaire chez les patients infectés par le VIH-1 comme les cellules Natural Killer ou les cellules T $CD8^{+}$ cytotoxiques.

# 11- Résultats concernant les études sur l'IL-18

## 11-1 Effet de l'IL-18 sur l'homéostasie des cellules NK lors de l'infection

Les résultats obtenus lors de cette étude concernant le rôle de l'IL-18 sur l'homéostasie des cellules Natural Killer chez les patients infectés par le VIH-1 sont présentés sous le format d'un article de recherche. Cet article a été soumis à un processus de révision par les pairs avant sa publication.

**Article 6 : Iannello A**, Samarani S, Debbeche O, Ahmad R, Boulassel MR, Tremblay C, Toma E, Routy JP and Ahmad A. Potential role of IL-18 in the immunopathogenesis of AIDS: involvement in fratricidal killing of NK cells. 2009. ***Journal of Virology* 83(12): 5999-6010**.

**Résumé :** Plusieurs études ont démontré que les concentrations circulantes d'IL-18 étaient élevées chez les patients infectés par le VIH-1 par rapport aux personnes non infectées. Lors de cette étude, nous avons investigué les conséquences de ces niveaux élevés d'IL-18 sur l'homéostasie des cellules Natural Killer chez ces patients. Nous avons observé une corrélation inverse entre les niveaux d'IL-18 et le nombre des différents sous-types de cellules NK chez les patients infectés par le VIH-1. Nous avons démontré que le traitement des cellules NK avec de l'IL-18 recombinante, lors de microculture *in vitro*, augmente la mort des cellules NK primaires et de la lignée cellulaire NK92. Cette mort fratricide est dépendante de l'interaction entre les protéines Fas/FasL et de la sécrétion de TNF-α. Nous avons montré que l'IL-18 augmente la sécrétion de TNF-α, induit l'expression de FasL sur les cellules NK et augmente la transcription du gène codant pour la protéine FasL. De plus, l'IL-18 réduit l'expression de la protéine anti-apoptotique Bcl-$X_L$ dans les cellules NK, ce qui augmente la sensibilité de ces dernières à un stimuli apoptotique médié par la voie Fas/FasL. L'ensemble de ces données suggèrent donc que les haut niveaux d'IL-18 circulantes présents chez les patients infectés contribuent à l'immunopathogénèse associée au SIDA en atérant l'homéostasie des cellules NK.

# Article 6

**Iannello A**, Samarani S, Debbeche O, Ahmad R, Boulassel MR, Tremblay C, Toma E, Routy JP and Ahmad A. Potential role of IL-18 in the immunopathogenesis of AIDS: involvement in fratricidal killing of NK cells. 2009. ***Journal of Virology*** **83(12): 5999-6010**.

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# Potential Role of Interleukin-18 in the Immunopathogenesis of AIDS: Involvement in Fratricidal Killing of NK Cells[▽]

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**We had shown earlier that the concentrations of circulating interleukin-18 (IL-18) are increased significantly in human immunodeficiency virus (HIV)-infected persons compared to HIV-seronegative healthy subjects. In the present study, we investigated the consequences of these elevated levels of IL-18 on natural killer (NK) cells and the immunopathogenesis of AIDS. We show here an inverse correlation between IL-18 concentrations and absolute numbers of various subsets of NK cells in infected persons. Recombinant human IL-18 caused increased death of a human NK cell line, as well as of primary human NK cells in vitro. The IL-18-mediated cell death was dependent upon Fas-FasL interactions and tumor necrosis factor alpha. IL-18 induced the expression of FasL on NK cells, increased the transcription from the human FasL promoter, reduced the expression of Bcl-$X_L$ in NK cells, and increased their sensitivity to FasL-mediated cell death. These results suggest that increased IL-18 concentrations present in the circulation of HIV-infected persons contribute to the immunopathogenesis of AIDS by altering NK cell homeostasis.**

Interleukin-18 (IL-18), originally named as the gamma interferon (IFN-γ)-inducing factor, is a multifunctional and pleiotropic cytokine with proinflammatory properties (24, 25, 27; reviewed in reference 10). The cytokine is a member of the IL-1 family and is produced mainly by monocytes, macrophages, dendritic cells, keratinocytes, enterocytes, Kupffer cells, adrenal cortex, adipose tissues, and neurohypophysis in the human body. The cells and tissues constitutively produce IL-18 and enhance its production in response to stress, infection, lipopolysaccharide, cold, and stimulation via Toll-like receptors. Since IL-18 induces production of IFN-γ from T and natural killer (NK) cells, it was regarded as a cytokine that promotes Th1 type immune responses. However, the cytokine promotes Th2 type responses in the absence of IL-12 by inducing production of IL-4 from mast cells and eosinophils. Therefore, depending upon the context, the cytokine can promote both Th1 and Th2 type immune responses (reviewed in references 26 and 37). IL-18 exerts its biological effects by binding with a unique receptor called IL-18 receptor (IL-18R), which is a heterodimer of α and β chains, both of which carry Toll–IL-1 receptor domains in their intracytoplasmic regions. Given that IL-18 is a proinflammatory cytokine, it is not surprising that increased concentrations of this cytokine have been reported in many chronic inflammatory conditions in humans, e.g., rheumatoid arthritis, multiple sclerosis, Crohn's disease, graft-versus-host disease, atherosclerosis, etc. (10).

Human immunodeficiency virus type 1 (HIV-1) is the etiological agent of AIDS in humans. The infections with this virus are usually accompanied by changes in the production of several immunologically important cytokines, e.g., IL-15, tumor necrosis factor alpha (TNF-α), IL-4, IL-12, IL-10, transforming growth factor β1, etc. (1, 5, 8; reviewed in references 7, 16, and 20). These cytokine disturbances play an important role in the immunopathogenesis of AIDS in HIV-infected persons. Concerning IL-18, we and others have demonstrated increased concentrations of this cytokine in HIV-infected persons (3, 30, 33; see reference 32 for a review). The simian immunodeficiency virus (SIV), which is a close cousin of HIV-1, also induces IL-18 production in rhesus monkeys (12).

NK cells constitute an important cellular component of innate immunity. Not only do they kill virus-infected cells, they also kill these cells via antibody-dependent cell-mediated cytotoxicity (4, 16). Activated NK cells have been associated with protection from HIV infection, as well as with delaying its progression toward AIDS. However, NK cell functions become compromised in HIV-infected AIDS patients. Furthermore, the absolute numbers and percentages of various NK cell subsets are decreased overtime in these patients (reviewed in references 11 and 15). It has been demonstrated that increased serum concentrations of IL-18 correlate inversely with NK cell numbers in the patients suffering from chronic inflammatory conditions (22, 29). In the present study, we sought to determine whether such a correlation also existed between the serum concentrations of this cytokine and NK cell numbers in HIV-infected individuals. We show here a significant inverse correlation between IL-18 serum concentrations and NK cell numbers in HIV-infected AIDS patients. We also provide ex-

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perimental evidence to show that IL-18 causes fratricidal cell death in human NK cells by inducing increased expression of FasL and TNF-α from these cells. The cytokine increases transcriptional activity of the human FasL gene promoter. Our study unravels the molecular mechanism underlying the inverse correlation between IL-18 levels and NK cell numbers and functions observed in chronic inflammatory conditions. Collectively, our studies demonstrate how increased IL-18 concentrations may be contributing to the immunopathogenesis of AIDS in HIV-infected persons by compromising NK cell responses.

## MATERIALS AND METHODS

**Cell culture.** All cells used in the present study were cultured at 37°C in 5% $CO_2$ humidified atmosphere. An IL-2-dependent NK cell line, NK92, was used in the present study. The cell line was established from a patient with rapidly progressing non-Hodgkin's lymphoma (ATCC; catalogue no. CRL-2407). NK92 cells were maintained in α-MEM (Gibco, Burlington, Ontario, Canada) containing 12.5% fetal calf serum (FCS), 12.5% horse serum, 2 mM L-glutamate, 100 μg of penicillin/ml, and 100 μg of streptomycin/ml (all from Life Technologies, Burlington, Ontario, Canada) and supplemented with 100 U of IL-2 (Roche, Mississauga, Ontario, Canada)/ml. The erythroleukemia cell line K562 was maintained in the RPMI 1640 culture medium (Gibco) supplemented with 10% FCS, 2 mM L-glutamate, 100 μg of penicillin/ml, and 100 μg of streptomycin/ml (hereafter referred to as culture medium). These cells are frequently used as target cells in measuring NK cell-mediated cytotoxicity by human peripheral blood mononuclear cells (PBMC). The PBMC were obtained from the peripheral blood from HIV-infected AIDS patients and HIV-seronegative healthy subjects. For this purpose, blood was collected in heparinized vacuum tubes, and PBMC were isolated by centrifugation over Ficoll-Hypaque (Pharmacia, Montreal, Quebec, Canada) as described earlier (3). The buffy coat at the interface of Ficoll-Hypaque and plasma was collected, washed, and resuspended in RPM1 1640 medium containing 10% FCS, 2 mM L-glutamate, 100 μg of penicillin/ml, and 100 μg of streptomycin/ml as described previously (3).

**Antibodies and recombinant cytokines.** The antibodies used in the present study were purchased: mouse anti-human FasL from BD Biosciences (Mississauga, Ontario, Canada); phycoerythrin (PE)-conjugated mouse anti-human FasL, mouse anti-human TNF-α, and its fluorescein isothiocyanate (FITC)-conjugated version from eBioscience (San Diego, CA); rabbit anti-human Bcl-$X_L$ from Cell Signaling Technology (Boston, MA); mouse anti-human Bcl-2 from Calbiochem (San Diego, CA); mouse anti-human GAPDH (glyceraldehyde-3-phosphate dehydrogenase) from Ambion (Austin, TX); and allophycocyanin (APC)- or PE-conjugated mouse anti-human CD3, APC-conjugated mouse anti-CD56, FITC-conjugated mouse anti-human CD16 and PE-conjugated mouse anti-human TRAIL, and isotype-matched antibodies from Biolegend (San Diego, CA). The recombinant human IL-18 used for the present study was purchased from MBL (Woburn, MA).

**Patients and sera collection.** Twenty-six HIV-infected viremic AIDS patients and an equal number of age-matched HIV-seronegative healthy subjects were recruited for the present study. The study was approved by the Institutional Ethics Committee. Peripheral blood samples were obtained from the study participants after we obtained their written informed consent. All of the patients had one or more AIDS-defining conditions and were receiving highly active antiretroviral therapy that comprised at least one protease inhibitor (saquinavir, ritonavir, or indinavir) and one or two reverse transcriptase inhibitors (stavudine, lamivudine, zidovudine, or didanosine). Detailed clinical and virological parameters of the patients are shown in Table 1. Serum was obtained from each blood sample after it was allowed to clot at room temperature for 2 to 3 h. Each serum sample was divided into aliquots and stored at −80°C until used.

**Measuring cytokine concentrations.** The concentrations of IL-18 were determined in serum samples by using commercial enzyme-linked immunosorbent assay (ELISA) kits from Bender Medsystems (Burlingame, CA). The detection limit for the kit was 12 pg/ml. Soluble TNF-α in the culture supernatants was measured by a commercial ELISA kit (eBioscience, San Diego, CA) with minimum detection limit of 4 pg/ml.

**NK cell microcultures.** For NK cell microculture experiments, $10^5$ NK92 or primary NK cells were resuspended in 200 μl of the culture medium containing 5 U of IL-2/ml in triplicate in the wells of a round-bottom 96-well plate. NK cells were obtained from PBMC by negative selection using a kit from Stem Cell Technology (Vancouver, British Columbia, Canada). Recombinant human IL-18

TABLE 1. Characteristics of 26 patients in this study

| Patient | CD4 (no. of cells/μl)[a] | Viral load ($\log_{10}$)[b] | Clinical stage[c] | Duration[d] |
|---|---|---|---|---|
| 1 | 334 | 2.70 | C2 | >4Y |
| 2 | 172 | 3.59 | C3 | >3Y |
| 3 | 497 | 2.70 | C1 | ND |
| 4 | 297 | 3.47 | C2 | 4Y 6M |
| 5 | 187 | 4.65 | C1 | 5Y 7M |
| 6 | 190 | 2.70 | C3 | ND |
| 7 | 254 | 4.18 | C3 | ND |
| 8 | 242 | 3.80 | C1 | >6Y |
| 9 | 299 | 2.70 | B1 | 6Y |
| 10 | 16 | 4.55 | C3 | 3Y |
| 11 | 165 | 2.70 | C1 | ND |
| 12 | 476 | 2.70 | B2 | 8Y 8M |
| 13 | 119 | 3.29 | C1 | ND |
| 14 | 171 | 4.79 | C3 | 4Y |
| 15 | 746 | 2.80 | C1 | ND |
| 16 | 26 | 3.80 | C3 | 4Y |
| 17 | 578 | 4.11 | C1 | >3Y |
| 18 | 164 | 2.80 | C1 | >3Y |
| 19 | 489 | 3.20 | C1 | 5Y |
| 20 | 519 | 2.70 | C1 | >5Y |
| 21 | 299 | 4.54 | C2 | 6Y |
| 22 | 176 | 2.70 | C1 | 6Y |
| 23 | 55 | 4.76 | C1 | ND |
| 24 | 38 | 5.34 | C3 | 3Y |
| 25 | 72 | 3.58 | C3 | 3Y |
| 26 | 643 | 2.80 | C1 | ND |

[a] Mean ± standard error, 278 ± 203; median, 216; range, 16 to 746.
[b] Mean ± standard error, 3.53 ± 0.85; median, 3.38; range, 2.70 to 5.34.
[c] The clinical stages are as described in reference 19.
[d] Y, year(s); M, month(s); ND, not determined.

was added to the microcultures at different concentrations with or without different neutralizing antibodies (described for each experiment individually). At different time points, the cells were washed with phosphate-buffered saline (PBS; pH 7.2) and were analyzed for different parameters.

**Western blotting.** The expression of different proteins was analyzed by Western blotting as described in our earlier publications (3). Briefly, $5 \times 10^6$ NK cells were incubated in the culture medium with or without treatment as detailed in individual experiments. At different time points after the incubation, the cells were washed with PBS and lysed in a lysis buffer containing Tris-HCl (pH 6.8; 50 mM), sodium dodecyl sulfate (SDS; 2%), leupeptin (1 mg/ml), phenylmethylsulfonyl fluoride (1 mM), and pepstatin (1 mg/ml). The lysates were clarified by centrifugation at $14,000 \times g$ for 15 min. Protein concentrations were determined in the lysates by using a commercial kit (Pierce, Inc., Nepean, Ontario, Canada). Portions (40 μg) of the lysate proteins were mixed with 2× SDS-polyacrylamide gel electrophoresis sample loading buffer containing 1 mM dithiothreitol, boiled, run on SDS–12% polyacrylamide gel electrophoresis gels, and electroblotted onto polyvinylidene difluoride membranes (Immobilon-P; Millipore, Ontario, Canada). After blockage of the membranes in 1% casein for 2 h at room temperature, they were incubated on a shaker with human protein-specific antibodies generated in mice, i.e., anti-FasL, anti-Bcl-$X_L$, anti-Bcl-2, or anti-GAPDH, at 4°C overnight. The protein bands were revealed by autoradiography by using biotinylated goat anti-mouse antibodies and a commercial chemiluminescent kit (Vectastain ABC-AmP; Vector Laboratories, Burlington, CA). Individual bands on the X-ray films were quantified by densitometry.

**Flow cytometry.** For immunostaining, $10^6$ cells were incubated with fluorochrome-conjugated antibodies for 30 min on ice and washed three times with PBS containing 0.05% bovine serum albumin and 0.002% sodium azide. The washed cells were resuspended in 2% paraformaldehyde and analyzed by flow cytometry using FACSCalibur (BD Biosciences).

**Determination of NK cell cytotoxicity.** NK cell cytotoxicity was determined with a standard $^{51}$Cr-release assay as described in our earlier publications (2, 4). Briefly, $10^4$ $^{51}$Cr-labeled K562 cells were dispended in 100 μl of the culture medium in triplicate in the wells of a V-bottom 96-well plate. Then, $2 \times 10^5$ PBMC obtained from HIV-infected/AIDS patients were added in 100 μl of the culture medium to each well. This gave a target-to-effector cell ratio of 1:20. The microcultures were incubated for 16 h at 37°C in a humidified 5% $CO_2$ atmo-

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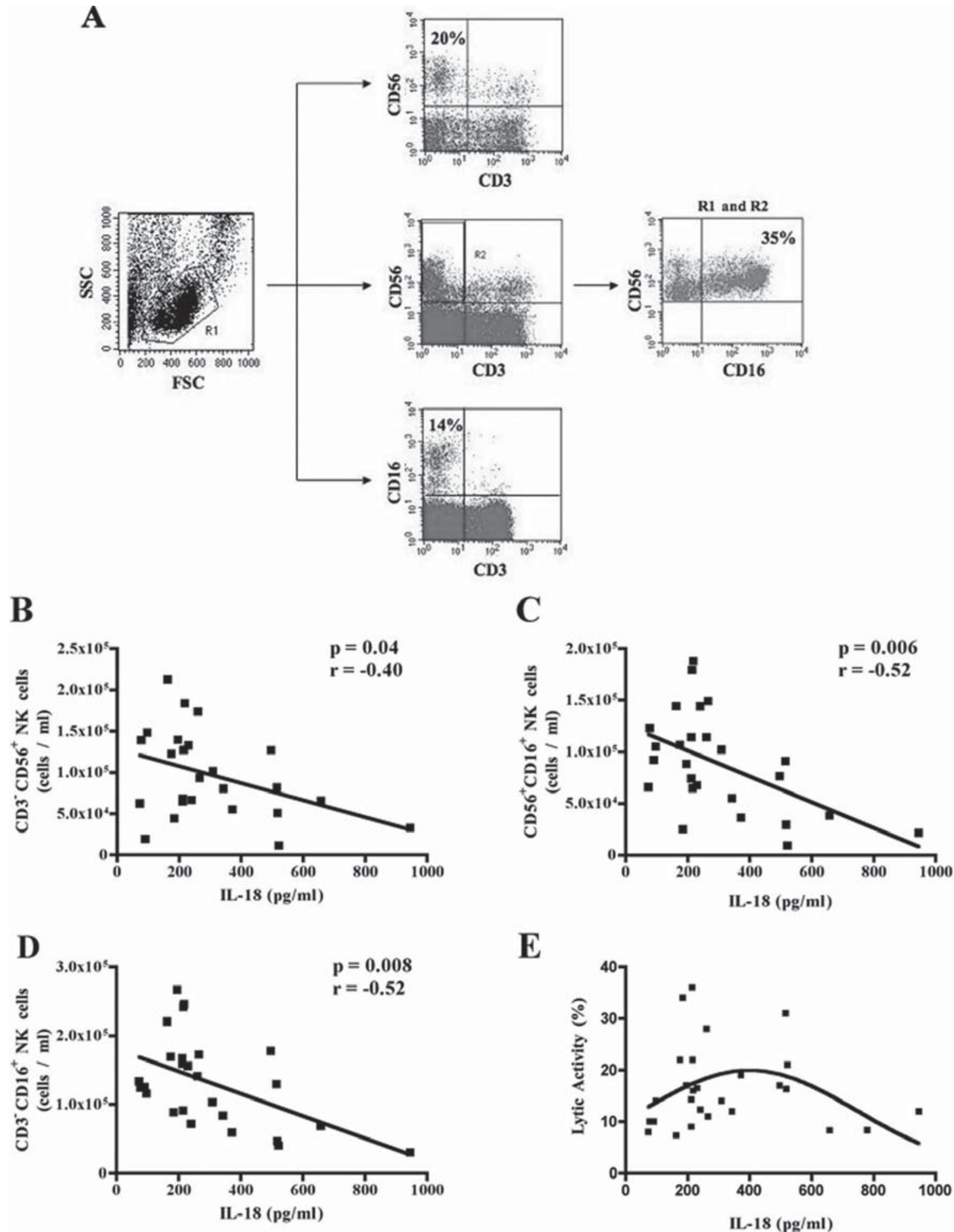


FIG. 1. Correlations of serum IL-18 levels with NK cell numbers and cytotoxicity. IL-18 concentrations in the sera of 26 HIV-infected persons were measured by a commercial ELISA kit, and the absolute numbers of different NK cells subsets present in their peripheral blood after gating on the viable lymphocyte population were determined by flow cytometry. (A) Representative flow cytometry data showing the gates used to identify the three different subsets of NK cells. The panel on the right shows results for $CD3^-$ $CD16^+$ $CD56^+$ NK cells. (B to D) Pearson correlations between serum concentrations of IL-18 and their absolute numbers of $CD3^-$ $CD56^+$ (B), $CD56^+$ $CD16^+$ (C), and $CD3^-$ $CD16^+$ (D) NK cells. The two parameters showed a statistically significant negative correlations ($P = 0.04$; $r = -0.40$ for panel B), ($P = 0.006$; $r = -0.52$ for panel C) and ($P = 0.008$; $r = -0.52$ for panel D). (E) Spontaneous NK cell activities of freshly isolated PBMC from these patients against K562 cells in a standard $^{51}$Cr-release assay. The serum IL-18 concentrations were plotted against NK cell-mediated cytotoxicities of the PBMC from HIV/AIDS patients. A bimodal effect of the serum IL-18 concentrations on NK cell-mediated cytotoxicities is evident.

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TABLE 2. Multivariate analysis between NK cell absolute numbers, IL-18 concentrations, and patient viral loads

| Control variable | Analysis[a] | Correlation between: CD3⁻ CD56⁺ NK cells and IL-18 concn | | | CD56⁺ CD16⁺ NK cells and IL-18 concn | | | CD56⁺ CD16⁺ NK cells and IL-18 concn | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | NK cells | IL-18 | Viral load | NK cells | IL-18 | Viral load | NK cells | IL-18 | Viral load |
| None | | | | | | | | | | |
| NK cell no. | Correlation | 1.000 | –0.404 | –0.070 | 1.000 | –0.523 | –0.012 | 1.000 | –0.507 | –0.0169 |
| | Significance | | 0.041 | 0.734 | | 0.006 | 0.955 | | 0.008 | 0.408 |
| | df | 0 | 24 | 24 | 0 | 24 | 24 | 0 | 24 | 24 |
| IL-18 concn | Correlation | –0.404 | 1.000 | –0.036 | –0.523 | 1.000 | –0.036 | –0.507 | 1.000 | –0.036 |
| | Significance | 0.041 | | 0.863 | 0.006 | | 0.863 | 0.008 | | 0.863 |
| | df | 24 | 0 | 24 | 24 | 0 | 24 | 24 | 0 | 24 |
| Viral load | Correlation | –0.070 | –0.036 | 1.000 | –0.012 | –0.036 | 1.000 | –0.0169 | –0.036 | 1.000 |
| | Significance | 0.734 | 0.863 | | 0.955 | 0.863 | | 0.408 | 0.863 | |
| | df | 24 | 24 | 0 | 24 | 24 | 0 | 24 | 24 | 0 |
| Viral load | | | | | | | | | | |
| NK cell no. | Correlation | 1,000 | –0.407 | | 1.000 | –0.524 | | 1.000 | –0.521 | |
| | Significance | | 0.043 | | | 0.007 | | | 0.008 | |
| | df | 0 | 23 | | 0 | 23 | | 0 | 23 | |
| IL-18 concn | Correlation | –0.407 | 1.000 | | –0.524 | 1.000 | | –0.521 | 1.000 | |
| | Significance | 0.043 | | | 0.007 | | | 0.008 | | |
| | df | 23 | 0 | | 23 | 0 | | 23 | 0 | |

[a] df, Degree of freedom. Significance refers to bilateral significance. Correlations are Pearson correlations.

sphere, after which 100 μl of the culture supernatants were collected from each well. The radioactivity released into these supernatants was counted by an automatic gamma counter (Clini Gamma; LKB Wallac, Finland). The percentage lysis of the target cells was determined as described earlier (2).

**Detection of cell death.** Dead cells were counted in microcultures by trypan blue exclusion. Apoptotic cells were detected by annexin V and propidium iodide (PI) staining using a commercial kit (BD Biosciences). Briefly, $10^5$ cells were washed in PBS and resuspended in 100 μl of 1× annexin V binding buffer. Cells were then stained with 5 μl of FITC-conjugated annexin V and 5 μl of PI for 15 min at room temperature in the dark. Cells were then diluted with 400 μl of 1× binding buffer and analyzed within 1 h after staining by flow cytometry using FACSCalibur (BD Biosciences).

**Transfection and FasL reporter gene assay.** Five million NK92 cells were transfected with a human FasL promoter-reporter gene construct. The construct (−511FasL pGL-3) contains a 511-bp region upstream the FasL gene start codon fused with the firefly luciferase gene in the plasmid pGL-3 basic (Promega) as described previously (23). The construct (5 μg of the DNA) was transfected transiently into the cells by using an Amaxa transfection system according to the accompanying protocol (Amaxa, Gaithersburg, MD). The transfected cells were divided into two equal aliquots at 12 h posttransfection. The cells were incubated in the culture medium for another 12 h with or without the addition of rhIL-18. The cells were washed and processed for luminescence detection using a luciferase reporter assay kit (Promega). The luminescence was analyzed by using a Mithras LB940 instrument (Berthold Technologies, Germany).

**Statistical analysis.** Group means were compared using Student *t* test and correlation between two parameters were determined by Pearson correlations using the software Prism (GraphPad, San Diego, CA). The multivariate analysis using partial Pearson correlations were determined with the software SPSS (SPSS, Inc., Chicago, IL). Differences and correlations were deemed significant at $P \leq 0.05$.

## RESULTS

**Levels of circulating IL-18 inversely correlate with NK cell numbers.** Increased IL-18 concentrations in the sera of patients suffering from chronic inflammatory conditions have been shown to be associated with loss of NK cell numbers and functionality (10, 22, 29). It is noteworthy that decreased NK cell numbers, as well as their decreased functionality, have been reported in HIV-infected AIDS patients. In order to determine whether increases in serum IL-18 levels were also associated with NK cell number and/or their cytolytic potential in HIV-infected persons, we measured IL-18 in the sera of HIV-infected persons by a commercial ELISA kit as described above and determined the absolute numbers of CD3⁻ CD56⁺, CD3⁻ CD16⁺, and CD56⁺ CD16⁺ NK cells present in their peripheral blood by flow cytometry. The staining and gating of the different NK cell subsets is shown in Fig. 1A. Furthermore, we measured spontaneous NK cell activities of freshly isolated PBMC of these patients against K562 cells in $^{51}$Cr-release assays as detailed in Materials and Methods. As shown in Fig. 1B to D, serum concentrations of IL-18 correlated negatively with absolute numbers of CD3⁻ CD56⁺ ($P = 0.04$; $r = -0.40$), CD3⁻ CD16⁺ ($P = 0.008$; $r = -0.52$), and CD16⁺ CD56⁺ NK cells ($P = 0.006$; $r = -0.52$). We determined whether the inverse correlation observed between IL-18 concentrations and NK cell numbers was influenced by viral loads of the patients. For this purpose, we performed a multivariate analysis between these three factors. Partial Pearson correlations were determined between IL-18 levels and NK cell numbers in controlling for viral loads. As shown in Table 2, the relationship between IL-18 concentrations and NK cell numbers in our cohort was independent of viral load.

Interestingly, the cytokine concentrations did not show a significant correlation with NK cell cytotoxicity. However, when we plotted the serum IL-18 concentrations against NK cell-mediated cytotoxicities of the PBMC, a bimodal effect of the cytokine was observed on the cytotoxic activities of the PBMC obtained from the patients (Fig. 1E). These data also suggest that at higher concentrations, IL-18 causes decreased NK cell-mediated cytotoxicity of the PBMC in HIV-infected patients. Collectively, these results show that, similar to other chronic inflammatory diseases, increased serum IL-18 concentrations are associated with diminished NK cell numbers. The findings also suggest that NK cell cytotoxic activities also start

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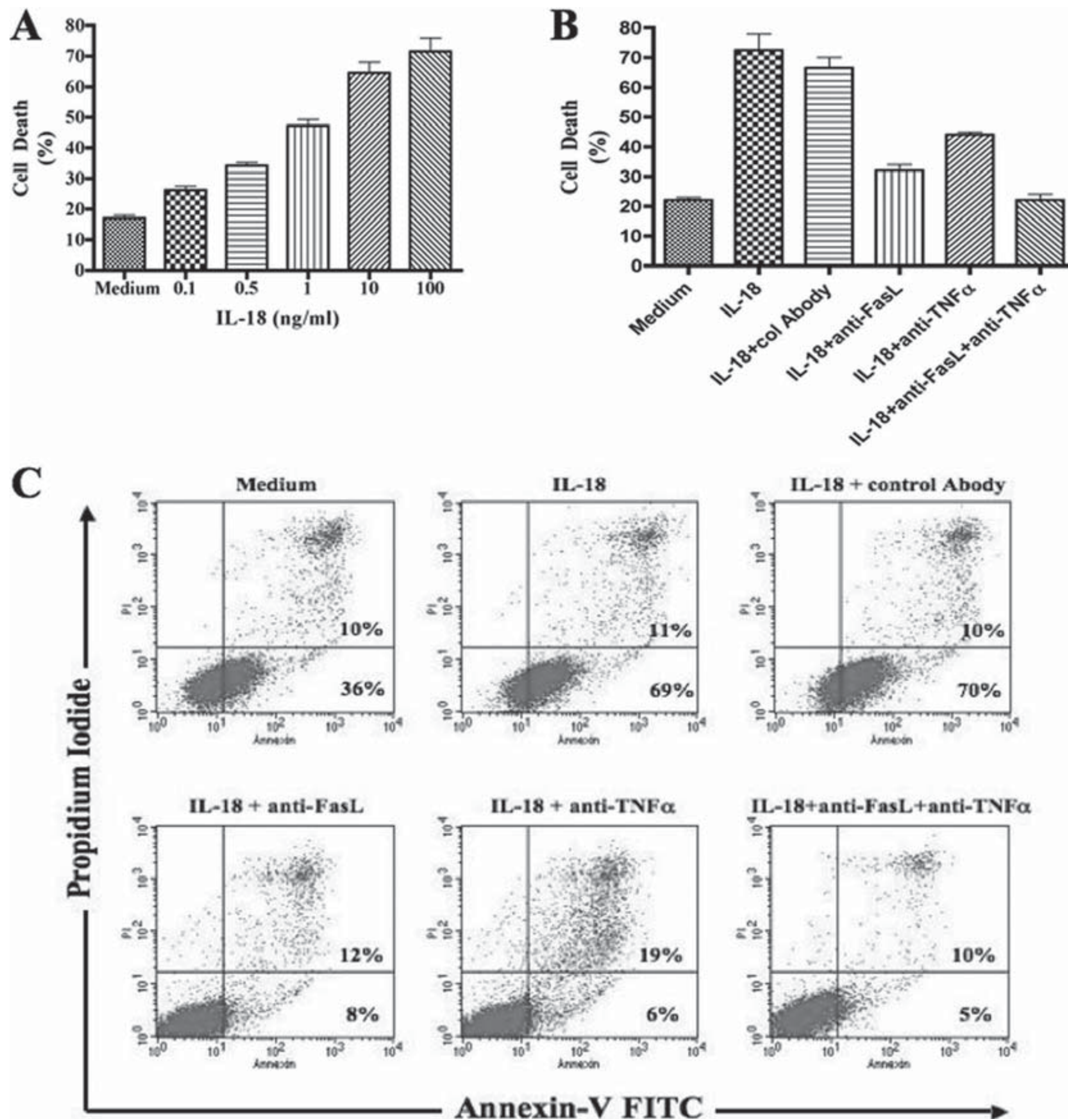


FIG. 2. IL-18 induces cell death in NK cells. (A) NK92 cells were cultured for 24 h with or without increasing concentrations of rhIL-18. The IL-18-mediated cell death was measured using a trypan blue exclusion assay. (B) NK92 cells were cultured for 24 h with or without rhIL-18 at a concentration of 10 ng/ml. Simultaneously, FasL-, TNF-α-, or TRAIL-neutralizing antibodies were added to the wells. (C) IL-18-mediated cell death was measured by using a trypan blue exclusion assay, as well as by staining with FITC-conjugated annexin V and PI.

declining progressively as the cytokine concentration increases beyond 400 pg/ml.

**IL-18 treatment induces fratricidal killing of NK cells.** In order to investigate the molecular mechanism(s) involved in this IL-18-mediated NK cell loss, we cultured the NK cell line NK92 in the presence of various concentrations of recombinant human IL-18. It is noteworthy that NK92 cells are in phase III clinical trials as therapeutic tools of cell therapy for different human disease conditions (6, 31). Using the trypan blue exclusion assay, we found that addition of the rhIL-18 resulted in significant death of NK cells compared to the cells cultured in the absence of the cytokine. These experiments were repeated several times. The results of a representative experiment are shown in Fig. 2A. Remarkably, the death was dose dependent. Since 10 ng of the cytokine/ml gave consistently more than 50% cell death (Fig. 2A and data not shown), we used this concentration in subsequent experiments. In order to determine whether the IL-18-mediated cell death was due to apoptosis, we stained the cells with FITC-conjugated annexin V and PI and analyzed them by flow cytometry. Again, the results of a representative of three experiments are shown in Fig. 2C. The culture of NK92 cells in the presence of rhIL-18 induced their increased binding to FITC-annexin V, which was reduced by an anti-FasL antibody.

In order to determine the molecular mechanism(s) underlying this IL-18-mediated apoptosis of NK cells, we performed experiments to determine whether this occurred via Fas/FasL, TNF-α/TNFR, and/or the TRAIL (TNF-related apoptosis-inducing ligand) pathways. For this purpose, we added anti-FasL antibodies, TNF-α neutralizing antibodies, and anti-TRAIL antagonist antibodies to NK92 cell cultures and determined their effects on the IL-18-mediated cell death. In these exper-

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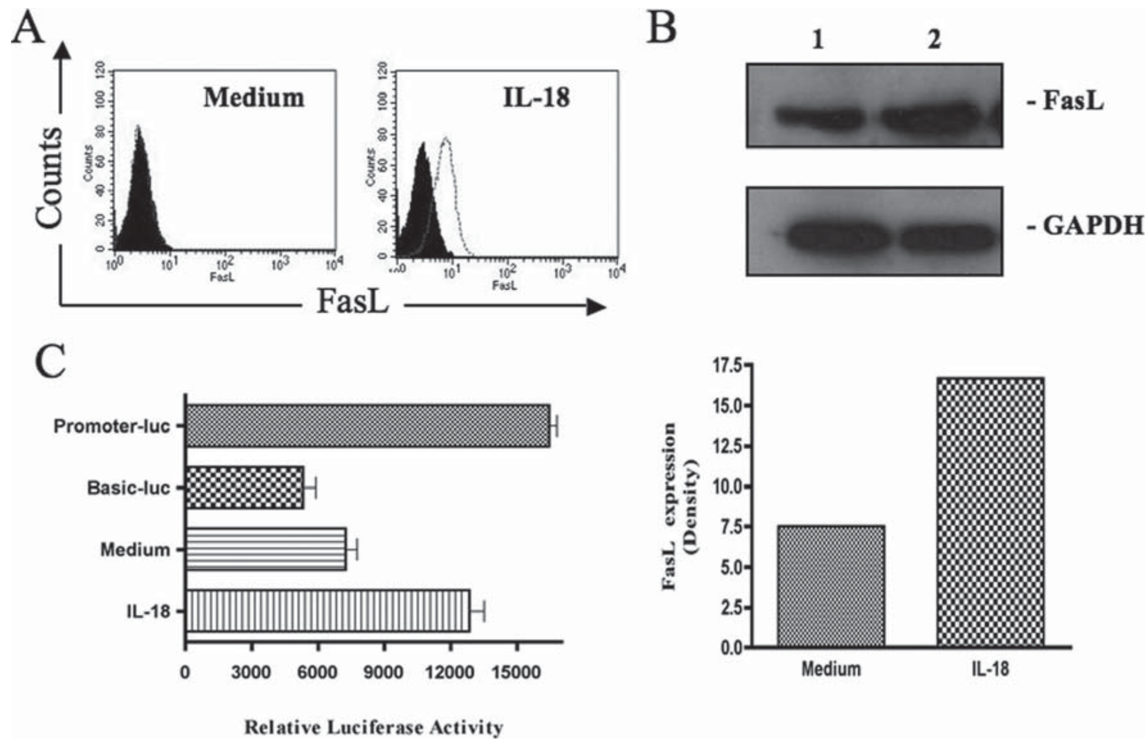


FIG. 3. IL-18 induces cell surface expression of FasL in NK cells. (A) NK92 cells were cultured for 20 h with or without rhIL-18 (10 ng/ml), stained for the surface-expressed FasL, and analyzed by flow cytometry. The black histogram show the signal obtained with the isotype-matched control antibody. (B) Cell lysates from NK92 cells cultured in medium (lane 1) or medium supplemented with 10 ng of rhIL-18/ml (lane 2) for 20 h were analyzed by Western blotting with a monoclonal anti-FasL antibody. (C) NK92 cells were transfected with the IL-18 promoter-firefly luciferase reporter gene construct. Twelve hours later, the transfected cells were divided into aliquots, followed by incubation in culture medium for another 12 h with or without the addition of rhIL-18. The expression of the reporter gene (luminescence) was measured in arbitrary units as described in Materials and Methods. Consistently similar results were obtained with three different transfections. Promoter-luc and Basic-luc represent positive and negative controls, respectively. The bars labeled Medium and IL-18 refer to the activity with the vector −511FasL pGL-3.

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iments, anti-FasL and anti-TNF-α antibodies significantly and consistently reduced apoptosis in the IL-18-added cultures compared to control antibodies. The results from a typical experiment are shown in Fig. 2B and C. Note a reduction by anti-FasL and anti-TNF-α to 20 and 25%;, respectively, versus 80% with control antibodies). Simultaneous addition of the anti-FasL and anti-TNF-α antibodies only slightly enhanced effect in the reduction of apoptosis (15% versus 20 and 25%). These results suggest that Fas-FasL and TNF-α–TNFR interactions were involved in the IL-18-mediated cell death in NK cell cultures. No reduction in the NK cells apoptosis was seen when anti-TRAIL antibodies were added in the NK cell cultures in the presence of rhIL-18 (data not shown). These results suggest that IL-18-mediated NK cell death does not involve TRAIL. This conclusion is further supported by the fact that NK92 cells do not express TRAIL and IL-18 does not induce its expression on these cells (data not shown).

**IL-18 induces cell surface expression of FasL on NK cells.** Since IL-18-mediated cell death involved Fas-FasL interactions, we sought to determine whether this cytokine induced expression of Fas and/or FasL on NK92 cells. For this purpose, we determined the expression of FasL by flow cytometry on NK92 cells after their cultures for 20 h with or without IL-18. In repeated experiments, we found that this NK cell line expresses little FasL on the cell surface constitutively and that IL-18 induces its expression on the cell surface (Fig. 3A). We also verified this increased expression of FasL by Western blots. As shown in Fig. 3B, the cell line expresses FasL constitutively intracellularly and rhIL-18 increases this expression after 20 h of treatment. Since FasL is known to be cleaved by certain metalloproteases into culture supernatants of FasL-expressing cells (18), we sought to determine whether IL-18-treated NK92 cells were secreting FasL in the culture media. For this purpose, we used a commercial ELISA kit to measure soluble FasL (sFasL) in the culture supernatants of these cells. No sFasL could be detected in the supernatants of NK92 cells cultured in the presence or absence of rIL-18 (data not shown). This result supports our unpublished data that the supernatants taken from IL-18-treated cells do not cause the death of NK92 cells. We next wanted to know whether IL-18 was inducing the expression of FasL at the gene expression level. For this purpose, we used a human IL-18 promoter-reporter gene construct as described in Materials and Methods. We transfected the construct into NK92 cells and determined the effect of IL-18 on the reporter gene expression. Our data (Fig. 3C) show that the FasL promoter activity is significantly increased in IL-18-treated cells compared to control untreated cells (12,850 ± 650 versus 7,250 ± 510 relative luciferase activity, respectively; $P < 0.01$). These data suggest that IL-18 increases FasL gene expression in NK92 cells.

**IL-18-rich sera from AIDS patients induces cell death and FasL expression in NK92 cells.** In an attempt to reproduce the

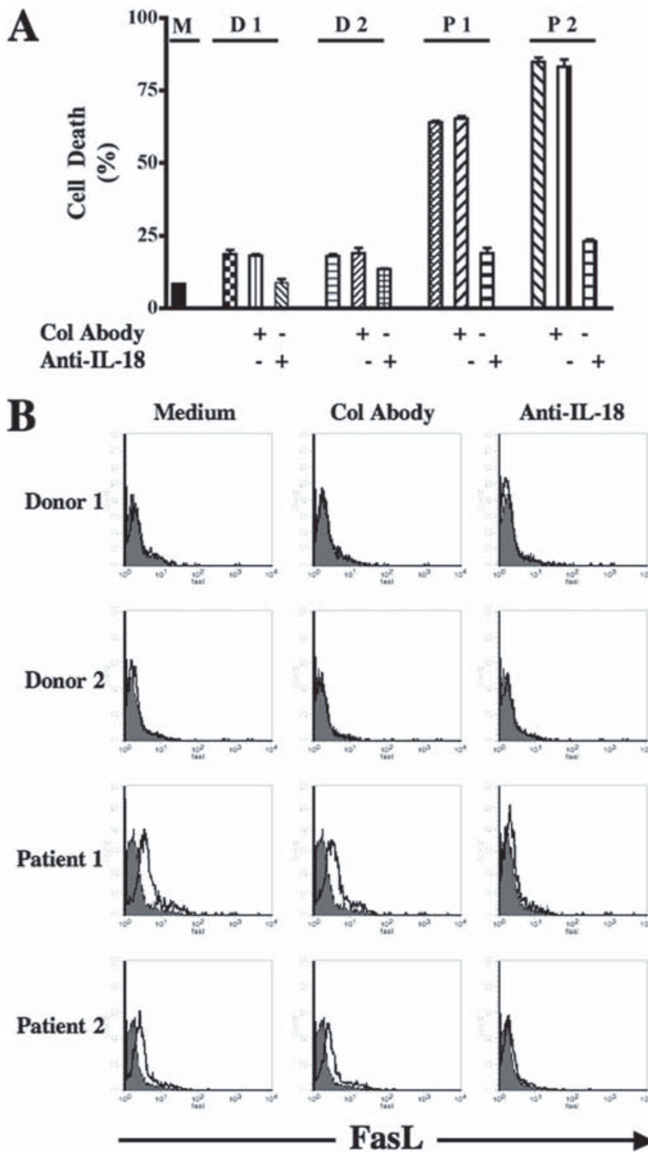


FIG. 4. Effect of IL-18-rich sera from AIDS patients on NK92 cells (A) NK92 cells were incubated for 24 h with medium only (M) or with IL-18-rich sera from AIDS patients (P1 and P2) and control donors (D1 and D2). These sera had been previously tested for their IL-18 contents (570 and 900 pg/ml for patients 1 and 2, respectively, and 73 and 63 pg/ml for donors 1 and 2, respectively). Simultaneously, neutralizing anti-IL-18 antibodies or control antibodies (mouse immunoglobulin G1) were added to the wells. After 24 h, the IL-18-mediated cell death was measured by counting dead cells using a trypan blue exclusion assay. (B) NK92 cells were incubated as described in panel A and stained for their surface expression of FasL by flow cytometry. Col Abody, control antibodies.

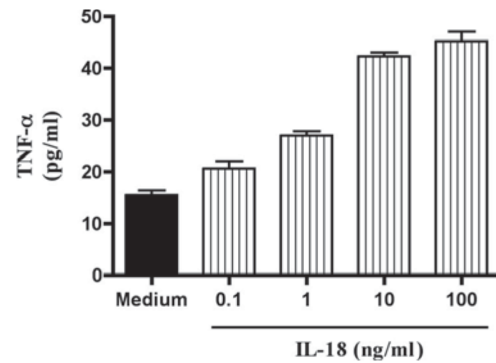


FIG. 5. IL-18 induces TNF-α in the culture supernatants of NK92 cells. NK92 cells were incubated for 24 h with increasing concentrations (0.1, 1, 10, and 100 ng/ml) of rhIL-18. After 24 h, the concentrations of TNF-α in the culture supernatants were measured by a commercial ELISA kit.

in vivo observation that increased IL-18 concentrations in the sera are associated with decreased NK cell numbers in AIDS patients under in vitro conditions, we incubated NK92 cells in sera from AIDS patients and control subjects. These sera had been previously tested for their IL-18 contents. In repeated experiments, we found that only the sera containing more than 500 pg of the cytokine/ml caused significant cell death ($P < 0.001$). The results from four representative sera are shown in Fig. 4A. The sera containing less than this concentration of the cytokine, irrespective of the fact whether they were from healthy or HIV-infected persons, did not cause significant cell death (data not shown). The addition of anti-IL-18 neutralizing antibodies in these cultures reduced cell death to the level of control sera. Furthermore, treatment of NK92 cells with these IL-18-rich sera induced the cell surface expression of FasL compared to sera from control subjects, and the presence of neutralizing anti-IL-18 antibodies in the culture abrogated the cell surface expression of FasL (Fig. 4B). Due to limited amounts of the sera containing more than 500 pg of IL-18/ml, the experiments were conducted with two samples. Taken together, these results confirm our earlier data obtained with the recombinant human IL-18.

**IL-18 induces TNF-α in the culture supernatants of NK92 cells.** Since anti-TNF-α antibodies inhibited IL-18-mediated cell death in NK92 cells, we determined whether IL-18 induced the secretion of TNF-α from these cells. For this purpose, we measured the concentration of TNF-α in the culture supernatants of the NK cells with or without treatment with IL-18. Figure 5 shows results from one of the two independent experiments. NK92 secreted low levels of this cytokine, and IL-18 treatment significantly ($P < 0.0001$) increased the amount of its secretion.

**IL-18 enhances susceptibility of NK92 cells to Fas/FasL-mediated apoptosis by downregulating Bcl-$X_L$ expression.** The supernatants from IL-18-treated cells were unable to induce cell death when added to NK92 cell cultures despite containing increased amounts of TNF-α. This suggested that IL-18 may not only kill NK92 cells by increasing the expression of FasL and TNF-α but also have increased the sensitivity of these cells to death-inducing stimuli. To investigate this possibility, we treated these cells with rhIL-18 for 12 h, washed them with the culture medium, and incubated them with equal amounts of anti-Fas agonist antibody. As shown in the Fig. 6A, the anti-Fas agonist causes more cell death in IL-18-treated NK92 cells than in cells that were not treated with the cytokine ($P = 0.0343$). These results are representative of three independent experiments. It is noteworthy that NK92 cells constitutively express Fas on their surface and rhIL-18 does not cause significant changes in the expression of this antigen in these cells (our unpublished data). Therefore, we used the same amount

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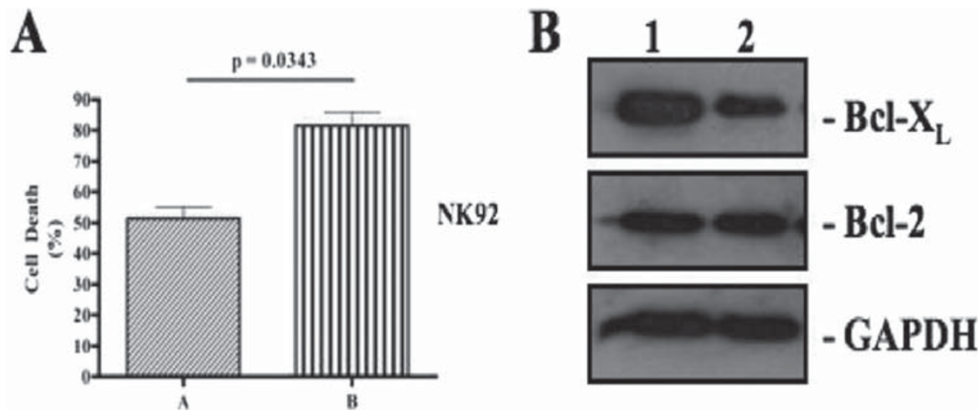


FIG. 6. IL-18 enhances susceptibility of NK92 cells to Fas/FasL-mediated apoptosis. (A) NK92 cells were cultured with the medium (lane A) or with the medium supplemented with 10 ng of rhIL-18/ml (lane B) for 12 h, washed, and incubated for another 12 h with 1 μg of anti-Fas agonistic antibody/ml. The dead cells were counted by using a trypan blue exclusion assay. The results are representative of two independent experiments. (B) Cell lysates from NK92 cells cultured in medium alone (lane 1) or in medium supplemented with 10 ng of rhIL-18/ml (lane 2) for 24 h were analyzed by Western blotting with anti-Bcl-2 monoclonal antibody and Bcl-$X_L$. A decrease in the expression of the latter protein in the IL-18-treated cell lysates is quite noticeable. The results are representative of three independent experiments.

of anti-Fas agonist antibody in these experiments to provide equal degrees of death-inducing stimuli. The results could be interpreted by assuming that IL-18 enhances the susceptibility of these cells to Fas/FasL-mediated cell death. The IL-18-induced susceptibility depended upon the dose of IL-18 used: the higher the dose, the more susceptible the cells become. We reasoned that one way of increasing this susceptibility could be by modulating the expression of one or more apoptosis-regulating proteins belonging to the Bcl-2 family in the IL-18-treated cells. In order to determine whether IL-18 treatment modulated the expression of any of these proteins, we performed Western blots for Bcl-2 and Bcl-$X_L$ on IL-18-treated and untreated NK92 cells. In repeated experiments, the cytokine downregulated the expression of Bcl-$X_L$, but not of Bcl-2, in this cell line. The data from a representative experiment are shown in shown in Fig. 6B.

**Recombinant human IL-18-induced changes in NK92 cells are recapitulated in primary human NK cells.** Finally, we wanted to know whether IL-18-mediated cell death in NK92 cells also occurs in primary human NK cells. For this purpose, we isolated NK cells from the peripheral blood of three donors and incubated them in the culture medium with different concentrations of rhIL-18. As shown in Fig. 7A, IL-18 also caused increased cell death in these cells in a dose-dependent manner ($P < 0.0001$). Furthermore, the death was prevented by both FasL and TNF-α neutralizing antibodies (Fig. 7B and C). In separate experiments, we determined the effects of the cytokine treatment on the expression of Fas and FasL on freshly isolated human NK cells. As shown in Fig. 8, the primary human NK cells, like NK92 cells, expressed little FasL on their surface, and IL-18 treatment induced this expression (Fig. 8A). Furthermore, the cells constitutively expressed Fas on their surface, and the cytokine treatment did not affect this expression in any significant manner (Fig. 8B). We also investigated whether IL-18 increased the susceptibility of primary NK cells to Fas-mediated apoptosis. For this purpose, we treated NK cells with IL-18 (10 ng/ml) from two different donors for 12 h separately and then added equal amounts of anti-Fas agonist antibodies. As shown in the Fig. 9A, the Fas agonistic antibodies caused significantly more ($P < 0.05$) death in IL-18-pretreated cells than in mock-treated cells in both donors. These data suggest that IL-18 also increases the susceptibility of primary human NK cells to Fas/FasL-induced death. Finally, we investigated the effects of IL-18 on the expression of Bcl-2 and Bcl-$X_L$ in primary human NK cells taken from two different donors. As shown in Fig. 9B, IL-18 treatment caused a decreased expression of Bcl-$X_L$, but not of Bcl-2, in primary human NK cells. Collectively, these results demonstrate that recombinant human IL-18-induced changes in the NK92 cell line are recapitulated in primary human NK cells.

## DISCUSSION

Our results show that, like other chronic inflammatory disease conditions (22, 29), there exists a negative correlation between NK cell numbers and the levels of the circulating IL-18 in HIV-infected persons. All major subsets of NK cells—i.e., $CD3^-$ $CD16^+$, $CD3^-$ $CD56^+$, and $CD16^+$ $CD56^+$ NK cells—showed almost equally strong inverse correlations with serum IL-18 levels in these patients. These data suggest that IL-18 is implicated in the loss of NK cell numbers in HIV-infected persons (see below), and all of these subsets of NK cells are equally susceptible to this adverse effect of this cytokine. Our results also shed light on the molecular mechanism involved in the IL-18-mediated loss of NK cells. Using an established human NK cell line, as well as primary human NK cells, we demonstrate that IL-18 induces fratricidal cell death in NK cells via two mechanisms: by inducing the expression of FasL and by increasing the production of TNF-α from NK cells. Our unpublished data show that NK92 cells, as well as freshly isolated primary human NK cells, constitutively express Fas on the cell surface, and IL-18 treatment does not increase Fas expression on these cells. However, the cytokine induces expression of FasL on the cell surface and TNF-α production from these cells. The two molecules induce cell death in NK cell cultures. Despite an increase in the expression of FasL, we were not able to detect soluble FasL from the NK cell supernatants cultured in the presence of rhIL-18. Furthermore,

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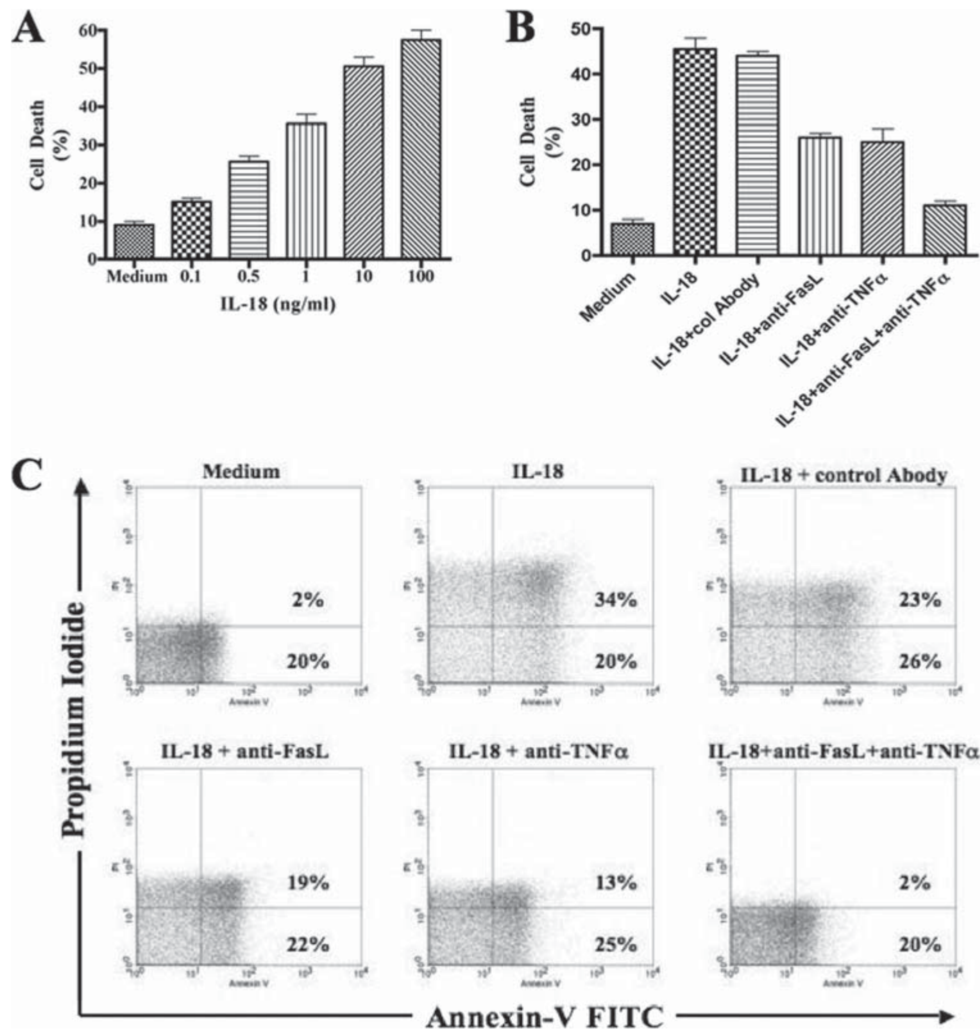


FIG. 7. IL-18 causes death in freshly isolated human NK cells. (A) Isolated primary NK cells from a healthy donor were cultured for 24 h with or without increasing concentrations of rhIL-18. IL-18-mediated cell death was measured by counting dead cells using a trypan blue exclusion assay. (B) Isolated human NK cells were cultured for 24 h in the presence of rhIL-18 (10 ng/ml) with or without the addition of anti-FasL antibodies or TNF-α- or TRAIL-neutralizing antibodies (1 μg/ml each). IL-18-mediated cell death was measured by counting dead cells using a trypan blue exclusion assay. (C) Human NK cells were treated as described above and stained with FITC-conjugated annexin V and PI and analyzed by flow cytometry. The results are representative of three independent experiments done on three different donors.

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these supernatants were not able to induce cell death when added to NK cell cultures (our unpublished data). We also noted that rhIL-18 caused an increase in the sensitivity of NK cells to apoptotic stimuli by decreasing the expression of the antiapoptotic protein, Bcl-$X_L$, in both NK92 and human NK cells. This may explain why the supernatants from the NK cells cultured in the presence of rhIL-18 (which are supposed to contain TNF-α) were not able to induce apoptosis of NK cells. A decreased expression of this antiapoptotic protein in rhIL-18-treated cells may be crucial for their apoptosis via FasL. To the best of our knowledge, this is the first report implicating IL-18 in causing the downregulation of an antiapoptotic protein in human NK cells. It will be interesting to determine whether the cytokine induces a similar downregulation of this protein in other immune cells, e.g., T cells.

We observed that rhIL-18 caused cell death in NK92 cells, as well as in primary human NK cells, in a dose-dependent manner. At concentrations as low as 100 pg/ml, the cytokine consistently caused significant cell death. However, when we tested sera from healthy and HIV-infected persons with known concentrations of IL-18, the sera containing more than 500 pg of IL-18/ml caused cell death. The sera containing lower concentrations of the cytokine, whether from HIV-infected or healthy persons, did not cause significant cell death. We believe that many other factors in the sera, e.g., IL-18BP, cytokines, growth factors, etc., may explain these differential effects.

It is noteworthy that this IL-18-mediated NK cell killing may represent a negative-feedback mechanism to control and terminate a proinflammatory immune response. It was demonstrated previously that combinations of two cytokines, IL-12 and IL-15, as well as IL-2 and IL-12, also cause death of human NK cells, which could be blocked by TNF-α neutralizing antibodies (28). The authors of that study did not implicate Fas-FasL interactions in this cytokine-mediated cell death. It is noteworthy that IL-18, but not IL-12 or IL-15, has been shown

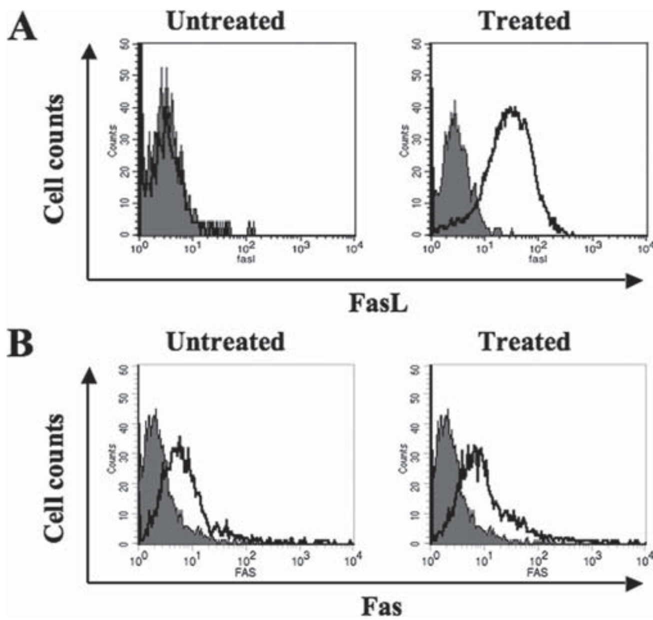


FIG. 8. Effect of rhIL-18 on cell surface expression of Fas and FasL on primary NK cells. Isolated human NK cells were incubated for 24 h with or without 10 ng of rhIL-18/ml, stained for the expression of FasL (A) and Fas (B) on their surfaces, and measured by flow cytometry. The results are representative of three independent experiments with three different donors.

to increase FasL-mediated cell death by murine NK cells and Th1 T-cell clones (9, 13, 36). However, the expression of FasL on NK cells was not measured in these studies. We show here for the first time that IL-18 induces FasL expression in a human NK cell line, as well as in freshly isolated primary human NK cells. Constitutively, these cells express very little of this antigen on the cell surface. We provide experimental evidence to show that in vitro the cytokine increases the transcription of the FasL gene via stimulating its promoter. It is quite conceivable that the cytokine-induced expression of FasL would result in upregulation of the FasL-mediated cytotoxicity of NK cells. However, these cells could also cause tissue destruction by killing Fas-positive body cells and tissues. Indeed, IL-18 has been implicated in FasL- and TNF-α-mediated liver damage by endotoxin in mice (34; reviewed in reference 35). It is noteworthy that cytokines such as IL-2, IL-12, IL-15, and IL-18 have the potential of enhancing innate and adaptive immunity against cancer and infectious diseases and are being used or considered both as adjuvants and as prophylactic and therapeutic tools. Our results suggest that careful consideration should be given to the in vivo use of these cytokines either alone or in different combinations due to their negative-feedback mechanisms and consequently their suppressive effects on immune responses.

Interestingly, one study (14) has shown that IL-18 prevents apoptosis of NK cells during their killing of target cells. The authors of that study ascribed this IL-18-mediated protection to enhanced expression of TRAF-1 and c-IAP. Another study (21) has shown that IL-18 induces expression of CD83, CCR7,

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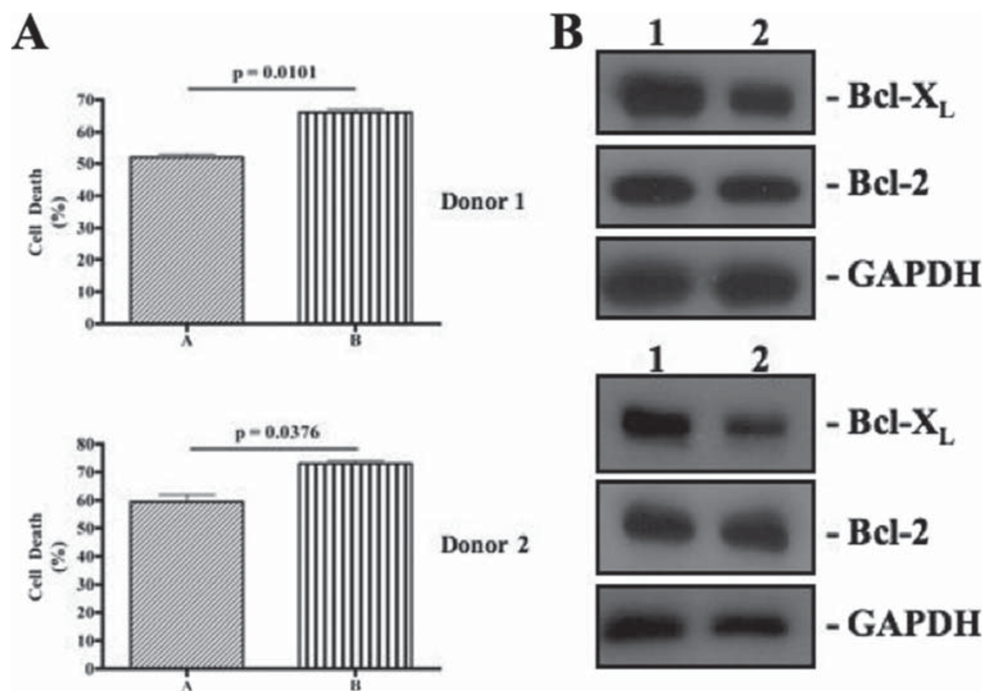


FIG. 9. IL-18 enhances the susceptibility of primary NK cells to Fas/FasL-mediated apoptosis. (A) Isolated primary NK cells from two donors were cultured with the medium (lane A) or with the medium supplemented with 10 ng of rhIL-18/ml (lane B) for 12 h, washed, and incubated for another 12 h with 1 μg of anti-Fas agonistic antibody/ml. The dead cells were counted by using a trypan blue exclusion assay. (B) Cell lysates from primary NK cells coming from two different donors were cultured in medium alone (lane 1) or in medium supplemented with 10 ng of rhIL-18/ml (lane 2) for 24 h and then analyzed by Western blotting with anti-Bcl-2 monoclonal antibody and Bcl-$X_L$. A decrease in the expression of the latter protein in the IL-18-treated cell lysates is notable.

and CD25 on NK cells and increases their migration to lymph nodes and interaction with other immunocytes (so-called helper function). It is noteworthy that our study examines NK cell death when they interact with each other after IL-18 treatment and does not contradict the results of these two studies. IL-18 increases FasL expression on NK cells and therefore IL-18-treated NK cells would kill Fas-positive target cells more efficiently. It is noteworthy that in our hands IL-18-treated NK92 did not cause enhanced killing of K562 cells, which lack Fas expression. Our results contradict those of Kalina et al. (17), who reported increased killing of these target cells by IL-18-treated NK92 cells. These differences may be attributed to different techniques used in the two studies: we used standard $^{51}$Cr-release assays, while the other study relied upon cell-staining dye PKH and PI intake. Furthermore, we cannot exclude the differential expression of Fas on the target cells.

It can be argued that our results may not be relevant since we used concentrations of IL-18 in several of our experiments that are much higher that those found in the circulation of healthy or HIV-infected persons. In fact, the concentrations of cytokines in the circulation represent a spillover from the cells and tissues that produce them. The precise concentrations of the cytokines in tissues where the cytokine-producing cells reside are not known. These concentrations are most likely to be much higher than those found in the circulation, the so-called “physiological” concentrations. Therefore, in the course of investigating basic biology of a cytokine, it is not inappropriate or “unphysiological” to perform experiments with cytokine concentrations higher than those found in the circulation. There are several examples in which researchers have used IL-18 concentrations in nanograms per milliliter (see, for example, references 17 and 21) and discovered certain unknown aspects of the cytokine biology.

In summary, the present study advances our understanding of the immunobiology of IL-18 and sheds light on how increased concentrations of this cytokine found in the circulation of HIV-infected patients may be contributing to AIDS pathogenesis.

## ACKNOWLEDGMENTS

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## REFERENCES

1. **Ahmad, A., R. Ahmad, E. Toma, R. Morisset, and J. Menezes.** 2000. Impaired induction of IL-15 in response to herpes simplex virus type 1 infection in peripheral blood mononuclear cells of HIV-infected patients. AIDS **14:** 744–746.
2. **Ahmad, A., R. Morisset, R. Thomas, and J. Menezes.** 1994. Evidence for a defect of antibody-dependent cellular cytotoxic (ADCC) effector function and anti-HIV gp120/41-specific ADCC-mediating antibody titres in HIV-infected individuals. J. Acquir. Immune Defic. Syndr. **7:**428–437.
3. **Ahmad, R., S. T. A. Sindhu, E. Toma, R. Morisset, and A. Ahmad.** 2002. Elevated levels of circulating interleukin-18 in human immunodeficiency virus-infected individuals: role of peripheral blood mononuclear cells and implications for AIDS pathogenesis. J. Virol. **76:**12448–12456.
4. **Ahmad, R., S. T. A. K. Sindhu, E. Toma, R. Morisset, J. Vincelette, J. Menezes, and A. Ahmad.** 2001. Evidence for a correlation between antibody-dependent cellular cytotoxicity-mediating anti-HIV-1 antibodies and prognostic predictors of HIV infection. J. Clin. Immunol. **21:**227–233.
5. **Allen, J., H. Wong, P. Guyre, G. Simon, and S. Wahl.** 1991. Association of circulating receptor Fcγ RIII-positive monocytes in AIDS patients with elevated levels of transforming growth factor-beta. J. Clin. Investig. **87:**1773–1779.
6. **Arai, S., R. Meagher, M. Swearingen, H. Myint, E. Rich, J. Martinson, and H. Klingemann.** 2008. Infusion of the allogeneic cell line NK-92 in patients with advanced renal cell cancer or melanoma: a phase I trial. Cytotherapy **10:**625–632.
7. **Cease, K. B., and J. A. Berzofsky.** 1994. Toward a vaccine for AIDS: the emergence of immunobiology-based vaccine development. Annu. Rev. Immunol. **12:**923–989.
8. **Chehimi, J., S. Starr, I. Frank, A. D’Andrea, X. Ma, R. MacGregor, J. Sennelier, and G. Trinchieri.** 1994. Impaired interleukin 12 production in human immunodeficiency virus-infected patients. J. Exp. Med. **179:**1361–1366.
9. **Dao, T., K. Ohashi, T. Kayano, M. Kurimoto, and H. Okamura.** 1996. Interferon-gamma-inducing factor, a novel cytokine, enhances Fas ligand-mediated cytotoxicity of murine T helper 1 cells. Cell. Immunol. **173:**230–235.
10. **Dinarello, C.** 2007. Interleukin-18 and the pathogenesis of inflammatory diseases. Semin. Nephrol. **27:**98–114.
11. **Fauci, A. S., D. Mavilio, and S. Kottilil.** 2005. NK cells in HIV infection: paradigm for protection or targets for ambush. Nat. Rev. Immunol. **5:**835–843.
12. **Giavedoni, L. D., M. C. Velasquillo, L. M. Parodi, G. B. Hubbard, and V. L. Hodara.** 2000. Cytokine expression, natural killer cell activation, and phenotypic changes in lymphoid cells from rhesus macaques during acute infection with pathogenic simian immunodeficiency virus. J. Virol. **74:**1648–1657.
13. **Hashimoto, W., T. Osaki, H. Okamura, P. D. Robbins, M. Kurimoto, S. Nagata, M. T. Lotze, and H. Tahara.** 1999. Differential antitumor effects of administration of recombinant IL-18 or recombinant IL-12 are mediated primarily by Fas-Fas ligand- and perforin-induced tumor apoptosis, respectively. J. Immunol. **163:**583–589.
14. **Hodge, D. L., J. J. Subleski, D. A. Reynolds, M. D. Buschman, W. B. Schill, M. W. Burkett, A. M. Malyguine, and H. A. Young.** 2006. The proinflammatory cytokine interleukin-18 alters multiple signaling pathways to inhibit natural killer cell death. J. Interferon Cytokine Res. **26:**706–718.
15. **Iannello, A., O. Debbeche, S. Samarani, and A. Ahmad.** 2008. Antiviral NK cell responses in HIV infection: I. NK cell receptor genes as determinants of HIV resistance and progression to AIDS. J. Leukoc. Biol. **84:**1–26.
16. **Iannello, A., O. Debbeche, S. Samarani, and A. Ahmad.** 2008. Antiviral NK cell responses in HIV infection. II. viral strategies for evasion and lessons for immunotherapy and vaccination. J. Leukoc. Biol. **84:**27–49.
17. **Kalina, U., D. Kauschat, N. Koyama, H. Nuernberger, K. Ballas, S. Koschmieder, G. Bug, W.-K. Hofmann, D. Hoelzer, and O. G. Ottmann.** 2000. IL-18 activates STAT3 in the natural killer cell line 92, augments cytotoxic activity, and mediates IFN-γ production by the stress kinase p38 and by the extracellular regulated kinases p44erk-1 and p42erk-21. J. Immunol. **165:** 1307–1313.
18. **Kayagaki, N., A. Kawasaki, T. Ebata, H. Ohmoto, S. Ikeda, S. Inoue, K. Yoshino, K. Okumura, and H. Yagita.** 1995. Metalloproteinase-mediated release of human Fas ligand. J. Exp. Med. **182:**1777–1783.
19. **Levy, J.** 1998. HIV and the pathogenesis of AIDS, p. 397. American Society for Microbiology, Washington, DC.
20. **Lotz, M., and P. Seth.** 1993. TGF beta and HIV infection. Ann. N. Y. Acad. Sci. **685:**501–511.
21. **Mailliard, R. B., S. M. Alber, H. Shen, S. C. Watkins, J. M. Kirkwood, R. B. Herberman, and P. Kalinski.** 2005. IL-18-induced CD83$^{+}$ CCR7$^{+}$ NK helper cells. J. Exp. Med. **202:**941–953.
22. **Mazodier, K., V. Marin, D. Novick, C. Farnarier, S. Robitail, N. Schleinitz, V. Veit, P. Paul, M. Rubinstein, C. A. Dinarello, J.-R. Harle, and G. Kaplanski.** 2005. Severe imbalance of IL-18/IL-18BP in patients with secondary hemophagocytic syndrome. Blood **106:**3483–3489.
23. **Mittelstadt, P. R., and J. D. Ashwell.** 1998. Cyclosporin A-sensitive transcription factor Egr-3 regulates Fas ligand expression. Mol. Cell. Biol. **18:** 3744–3751.
24. **Nakamura, K., H. Okamura, K. Nagata, T. Komatsu, and T. Tamura.** 1993. Purification of a factor which provides a costimulatory signal for gamma interferon production. Infect. Immun. **61:**64–70.
25. **Nakamura, K., H. Okamura, M. Wada, K. Nagata, and T. Tamura.** 1989. Endotoxin-induced serum factor that stimulates gamma interferon production. Infect. Immun. **57:**590–595.
26. **Nakanishi, K., T. Yoshimoto, H. Tsutsui, and H. Okamura.** 2001. Interleukin-18 regulates both TH1 and TH2 responses. Annu. Rev. Immunol. **19:** 423–474.
27. **Okamura, H., H. Tsutsui, T. Komatsu, M. Yutsudo, A. Hakura, T. Tanimoto, K. Torigoe, T. Okura, Y. Nukada, K. Hattori, K. Akita, M. Namba, F. Tanabe, K. Konishi, S. Fukuda, and M. Kurimoto.** 1995. Cloning of a new cytokine that induces IFN-γ production by T cells. Nature **378:**88–91.
28. **Ross, M. E., and M. A. Caligiuri.** 1997. Cytokine-induced apoptosis of human natural killer cells identifies a novel mechanism to regulate the innate immune response. Blood **89:**910–918.

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29. **Shibatomi, K., K. Ida, S. Yamasaki, T. Nakashima, T. Origuchi, A. Kawakami, K. Migita, Y. Kawabe, M. Tsujihata, P. Anderson, and K. Eguchi.** 2001. A novel role for interleukin-18 in human natural killer cell death: high serum levels and low natural killer cell numbers in patients with systemic autoimmune diseases. Arthritis Rheum. **44:**884–892.
30. **Song, W., C. M. Wilson, S. Allen, C. Wang, Y. Li, R. A. Kaslow, and J. Tang.** 2006. Interleukin 18 and human immunodeficiency virus type I infection in adolescents and adults. Clin. Exp. Immunol. **144:**117–124.
31. **Tam, Y., J. Martinson, K. Doligosa, and H. Klingemann.** 2003. Ex vivo expansion of the highly cytotoxic human natural killer-92 cell-line under current good manufacturing practice conditions for clinical adoptive cellular immunotherapy. Cytotherapy **5:**259–272.
32. **Torre, D., and A. Pugliese.** 2006. Interleukin-18: a proinflammatory cytokine in HIV-1 infection. Curr. HIV Res. **4:**423–430.
33. **Torre, D., F. Speranza, R. Martegani, A. Pugliese, F. Castelli, C. Basilico, and G. Biondi.** 2000. Circulating levels of IL-18 in adult and paediatric patients with HIV-1 infection. AIDS **14:**2211–2212.
34. **Tsutsui, H., K. Matsui, N. Kawada, Y. Hyodo, N. Hayashi, H. Okamura, K. Higashino, and K. Nakanishi.** 1997. IL-18 accounts for both TNF-alpha- and Fas ligand-mediated hepatotoxic pathways in endotoxin-induced liver injury in mice. J. Immunol. **159:**3961–3967.
35. **Tsutsui, H., K. Matsui, H. Okamura, and K. Nakanishi.** 2000. Pathophysiological roles of interleukin-18 in inflammatory liver diseases. Immunol. Rev. **174:**192–209.
36. **Tsutsui, H., K. Nakanishi, K. Matsui, K. Higashino, H. Okamura, Y. Miyazawa, and K. Kaneda.** 1996. IFN-gamma-inducing factor up-regulates Fas ligand-mediated cytotoxic activity of murine natural killer cell clones. J. Immunol. **157:**3967–3973.
37. **Yoshimoto, T., and K. Nakanishi.** 2006. Roles of IL-18 in basophils and mast cells. Allergy Int. **55:**105–113.


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### 11-2 Déséquilibre entre l'IL-18 et l'IL-18BP lors de l'infection

Les résultats obtenus lors de cette étude concernant la régulation de l'IL-18 et de son antagoniste l'IL-18BP sur la réplication du VIH-1 sont présentés sous le format d'un article de recherche. Cet article a été soumis à un processus de révision par les pairs avant sa publication.

**Article 7 : Iannello A**, Boulassel MR, Samarani S, Tremblay C, Toma E, Routy JP and Ahmad A. HIV causes imbalance in production of interleukin-18 and its natural antagonist in HIV-infected AIDS patients: implications for enhanced viral replication. 2009. ***Journal of Infectious Diseases*** **201(4): 608-17.**

**Résumé :** Avant cette étude, aucune information était détenue concernant la régulation de l'IL-18BP chez les patients infectés par le VIH-1. Nous avons donc réalisé une étude cross-sectionelle sur des sérums de patients infectés ou non par le VIH-1 lors de laquelle nous avons mesuré par ELISA les concentrations sériques d'IL-18 et d'IL-18BP. Nous avons infecté des macrophages *in vitro* par le VIH-1 afin de mesurer par ELISA la sécrétion d'IL-18 et d'IL-18BP. Nous avons de plus déterminé l'effet de l'IL-18 sur la réplication virale au niveau de cellules T $CD4^+$ infectées. Contrairement aux niveaux d'IL-18, les niveaux d'IL-18BP sont réduits dans les sérums de patients infectés par rapport aux personnes non infectées. Cette réduction résulte sur des niveaux élevés d'IL-18 libre dans leurs sérums. L'infection des macrophages par le VIH-1 augmente la sécrétion d'IL-18 et réduit celle d'IL-18BP. Nos études on aussi montré que l'IL-10 et le TGF-β, dont les concentrations sont élevées chez ces patients, permettent de réduire la production d'IL-18BP au niveau des macrophages sans infection ou d'une manière additive à l'infection. Nous avons aussi démontré que l'IL-18 augmente la réplication virale dans les cellules T $CD4^+$. Nos études démontrent un déséquilibre dans la production d'IL-18 et de son antagoniste chez les personnes infectées par le VIH-1. L'infection virale ainsi que le milieu cytokinique environnant les macrophages semblent donc être à l'origine du déséquilibre. L'augmentation de l'activité biologique de l'IL-18 qui en résulte pourrait ainsi augmenter la réplication du VIH-1 au niveau des cellules T $CD4^+$.

# <u>Article 7</u>

**Iannello A**, Boulassel MR, Samarani S, Tremblay C, Toma E, Routy JP and Ahmad A. HIV causes imbalance in production of interleukin-18 and its natural antagonist in HIV-infected AIDS patients: implications for enhanced viral replication. 2009. ***Journal of Infectious Diseases*** **201(4): 608-17.**

MAJOR ARTICLE

# HIV-1 Causes an Imbalance in the Production of Interleukin-18 and Its Natural Antagonist in HIV-Infected Individuals: Implications for Enhanced Viral Replication

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***Background.*** Concentrations of interleukin (IL)–18 increase in the circulation of human immunodeficiency virus (HIV)–infected persons. However, nothing is known concerning the regulation of IL-18–binding protein (IL-18BP), which neutralizes IL-18 in vivo. This issue is addressed in the present study.

***Methods.*** Serum samples obtained from healthy subjects and HIV-infected patients were analyzed by enzyme-linked immunosorbent assay to determine their IL-18 and IL-18BP contents. Human monocyte-derived macrophages (MDMs) were infected in vitro with HIV type 1 (HIV-1), and the production of these 2 cytokines by these cells was measured. Finally, we determined the effect of IL-18 on HIV-1 replication in human cells.

***Results.*** In contrast to IL-18 levels, IL-18BP levels decreased in the serum of HIV-infected patients. This decrease resulted in enhanced levels of free IL-18 in the serum of such patients. The infection increased production of IL-18 but decreased that of IL-18BP in MDMs. IL-10 and transforming growth factor–$\beta$, concentrations of which are increased in HIV-infected persons, also decreased production of IL-18BP by human MDMs. Finally, recombinant human IL-18 enhanced HIV-1 replication in human $CD4^+$ T cells.

***Conclusions.*** Production of IL-18 and its antagonist becomes imbalanced in HIV-1–infected persons. The infection and the cytokine milieu play a role in this decreased production. The increased biological activities of IL-18 may enhance viral replication in human $CD4^+$ T cells.

Interleukin (IL)–18 is a multifunctional and pleiotropic cytokine with unique proinflammatory, immune regulatory, proapoptotic, and atherogenic properties [1, 2]. In concert with IL-12, IL-18 acts as the most powerful inducer of interferon (IFN)–$\gamma$ in natural killer (NK) and T cells [1]. The cytokine is mainly produced by activated macrophages and several other types of cells in the body. In the circulation, IL-18 is bound specifically with a naturally occurring antagonist, IL-18–binding protein (IL-18BP). The bound IL-18 becomes functionally inactive and cannot transduce signals via IL-18–specific receptors. This protects the body from potentially harmful proinflammatory effects of the cytokine. Like IL-18, IL-18BP is constitutively produced from a wide variety of cells in the body, including macrophages. It has much higher affinity for IL-18, compared with that of IL-18 for IL-18–specific receptor [3–5]. The protein exists in humans in 4 different isoforms, designated as "a–d," which result from alternate splicing of the IL-18BP messenger RNA. Of these isoforms, the "a" isoform accounts for almost all IL-18–neutralizing activity of IL-18BP in the circulation [3].

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We and other investigators have shown that concentrations of IL-18 are significantly increased in the circulation of human immunodeficiency virus (HIV) type 1 (HIV-1)–infected individuals ([6–8]; reviewed in [9]). However, to the best of our knowledge, no information exists concerning production of IL-18BP in these individuals. This information is important for understanding regulation of the biological activity of IL-18 in HIV-1 infection. We addressed this issue in the present study. We also investigated the effects of HIV-1 infection on the production of these 2 soluble mediators by human monocyte-derived macrophages (MDMs). Increased biological activities of IL-18 in HIV-infected persons may be relevant to the infection, because we observed an enhancing effect of the cytokine on HIV-1 replication in human $CD4^+$ T cells.

## MATERIALS AND METHODS

***Generation of human MDMs.*** The MDMs were generated from the peripheral blood mononuclear cells (PBMCs), which were prepared from venous blood samples obtained from HIV-infected individuals and HIV-seronegative healthy subjects, as described elsewhere [6]. The cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 media that contained 10% fetal calf serum, 2 mmol/L L-glutamate, 100 $\mu$g/mL penicillin, and 100 $\mu$g/mL streptomycin at 37°C in a humidified atmosphere containing 5% $CO_2$. To generate MDMs, the monocytes present in the PBMCs were isolated by adherence to plastic dishes and were allowed to differentiate into macrophages for 5 days via culture in culture medium with 5% human AB-positive serum and 2 ng/mL recombinant human (rh) granulocyte-macrophage colony-stimulating factor.

***Antibodies and recombinant cytokines.*** The cytokines used in the present study included rhIL-2 from Roche; rhIL-18, rhIL-10, and rh granulocyte-macrophage colony-stimulating factor from Biosource; and rh transforming growth factor (TGF)–$\beta$ and rhIL-12 from eBioscience. Phytohemagglutinin was purchased from Sigma-Aldrich, and lamivudine (3TC) and p24 enzyme-linked immunosorbent assay (ELISA) kits were obtained from the AIDS Vaccine Program (National Cancer Institute–Frederick).

***Patients and collection of serum samples.*** Serum samples were obtained from 114 HIV-infected individuals and 32 age-matched healthy HIV-seronegative individuals. The characteristics of the infected individuals are presented in Table 1. The HIV infection was defined as a primary infection (PI), if $\leqslant$6 months had passed since the date of infection, and as chronic infection (CI), if >6 months had passed since this date. Individuals with CI who were receiving highly active antiretroviral therapy (HAART) were categorized as CIH patients. CIH patients who responded poorly to HAART and had detectable plasma viral loads were characterized as CIH viremic (CIH-V) patients. CIH patients who responded to HAART and had undetectable viral RNA in their plasma (<50 HIV-1 RNA copies/mL) were categorized as CIH aviremic (CIH-A) patients. All individuals in the CIH categories and 15 individuals in the CI category had $\geqslant$1 acquired immunodeficiency syndrome (AIDS)–defining condition. HAART consisted of different regimens that included $\geqslant$1 nucleoside and/or nonnucleoside reverse-transcriptase inhibitors in single, dual, or triple formulations, in combination with 1 or 2 protease inhibitors or a chemokine (C-C motif) receptor 5 (CCR-5) antagonist (Vicriviroc; Schering-Plough). The date of infection of each HIV-infected individual was estimated using his/her clinical and laboratory data as well as his/her medical history, as described elsewhere [10]. We followed the guidelines proposed by the Acute HIV Infection and Early Disease Research Program sponsored by the Division of AIDS of the National Institutes of Allergy and Infectious Disease.

**Table 1. Different Categories of Human Immuodeficiency Virus (HIV)–Infected Individuals**

| Category (no. of patients) | Clinical characteristic: $CD4^+$ T cell count, median (range), cells/$\mu$L of blood | Clinical characteristic: HIV-1 RNA load, median (range), copies/mL of plasma |
|---|---|---|
| Primary infection ($n$ = 30) | 381 (3–854) | 29,799 (156–511,143) |
| Chronic infection ($n$ = 37) | 303 (92–1261) | 32,949 (267–307,532) |
| CIH-V ($n$ = 32) | 236 (16–746) | 2400 (500–457,000) |
| CIH-A ($n$ = 15) | 519 (220–647) | <50 |

**NOTE.** CIH-A, chronically infected aviremic patients who were receiving highly active antiretroviral therapy (HAART); CIH-V, chronically infected patients with viremia who are receiving HAART; HIV-1, HIV type 1.

***Measurement of IL-18 and IL-18BP concentrations.*** Concentrations of IL-18 and IL-18BP in the serum samples and culture supernatants were determined using commercial ELISA kits from Bender Medsystems and R&D Systems, respectively. The detection limits for these kits are 12 pg and 60 pg/mL, respectively. The IL-18 kit measures total IL-18 (both free as well as IL-18BP–bound forms). The IL-18BP kit is specific for measurement of the "a" isoform, which accounts for almost all the IL-18–binding activity in the human circulation [3]. For each individual, free IL-18 was calculated from the individual's data on serum IL-18 and IL-18BP concentrations, in accordance with the law of mass action, as described elsewhere [11]. The calculation was based on the fact that IL-18BP binds IL-18 with a 1:1 stoichiometry and with a dissociation constant ($K_d$) of 0.4 nmol/L.

***HIV-1 infection of MDMs.*** In separate experiments, MDMs were infected (multiplicity of infection, 1) with a dual-tropic strain (89.6) or an M-tropic HIV-1 strain (YU-2) for 2 h at 37°C, and they were extensively washed. The cells were incubated at 37°C, and their culture supernatants were collected

after different lengths of time (detailed in individual experiments), clarified by centrifugation, aliquoted, and stored at −20°C until they were analyzed. In some experiments, the effects of different cytokines on the production of IL-18 and IL-18BP or on replication of HIV-1 in MDMs were determined. In these experiments, the cytokines were added to the cell cultures at equimolar concentrations (15 $\mu$mol/L) at the time of infection.

***Isolation and HIV-1 infection of $CD4^+$ T cells.*** To investigate the effect of IL-18 (either alone or in combination with other cytokines) on HIV-1 replication in $CD4^+$ T cells, we isolated these cells from the PBMCs of healthy HIV-seronegative donors. The PBMCs were activated with rhIL-2 (100 U/mL) and phytohemagglutinin (10 $\mu$g/mL) for 3 days at 37°C. $CD4^+$ T cells were isolated by negative selection performed using a kit (Stem Cell Technology). The purified $CD4^+$ T cells were >95% pure, as verified by flow cytometry (data not shown). The cells were infected (multiplicity of infection, 1) in vitro with HIV-1 (NL4.3) for 2 h at 37°C, and they were extensively washed with the culture medium to remove the residual virus. The cytokines (indicated in individual experiments) were added to the infected cell cultures immediately after the infection. The culture supernatants were collected after different lengths of time (as detailed in individual experiments), clarified by centrifugation, aliquoted, and stored at −20°C until they were analyzed to determine their HIV-1 p24 contents.

***Statistical analysis.*** The IL-18 and IL-18BP data from serum samples were analyzed using nonparametric one-way analysis of variance (Kruskal-Wallis test) and Dunn's multiple comparison test. The data from in vitro experiments were analyzed using classical one-way analysis of variance and the Tukey multiple comparison test. Pearson's correlation was used to find correlation between 2 variables. The software used was GraphPad software (version 4; Prism).

## RESULTS

***Increased IL-18 and decreased IL-18BP serum concentrations in HIV-infected individuals.*** As shown in Figure 1A, serum samples obtained from all groups of HIV-infected individuals had significantly ($P < .001$) increased levels of IL-18, compared with serum samples obtained from healthy subjects. The average IL-18 concentrations in the serum samples from infected individuals were ~2.5- to 3-fold higher than those in the serum samples from control individuals. Of interest, no significant differences ($P > .05$) were observed between the serum IL-18 concentrations in viremic and aviremic individuals receiving HAART. Results of analysis of the IL-18BP contents of these serum samples are shown in Figure 1*B*. Unlike IL-18, IL-18BP was present in significantly lower ($P < .001$) median concentrations in the serum samples obtained from HIV-infected persons than in the serum samples obtained from control individuals, with the exception of the CIH individuals. No statistically significant ($P > .05$) differences were observed within the infected groups, for these 2 parameters. However, the IL-18 concentration tended to increase in aviremic patients, and the IL-18BP concentration tended to increase in chronically infected patients (both viremic and aviremic) who were receiving HAART, compared with chronically infected patients who were not receiving HAART. No statistically significant correlations were found ($P > .05$) between IL-18 or IL-18BP levels and viral loads, $CD4^+$ T cell counts, or $CD8^+$ T cell counts in all groups of infected individuals (data not shown).

***Correlation between serum IL-18 and IL-18BP concentrations in healthy individuals but not in HIV-infected individuals.*** Because IL-18BP neutralizes IL-18 in vivo and protects body cells from its tissue-destructive effects, we reasoned that the concentrations of the 2 soluble mediators might be correlated with each other. As shown in Figure 1*C*, a significant positive correlation ($P < .001$; $r = 0.64$) was found between the 2 parameters in healthy persons. Of interest, no significant correlations were found between these 2 cytokines in the serum samples of HIV-infected individuals in all groups ($P = .32$ and $r = -0.18$ for patients with PI [Figure 1*D*], $P = .47$ and $r = -0.12$ for patients with CI [Figure 1*E*], $p = .58$ and $r = -0.1$ for CIH-V patients [Figure 1*F*], and $P = .41$ and $r = -0.23$ for CIH-A patients [Figure 1*G*]). These data suggest that coordinated production of these 2 soluble mediators, as observed in control subjects, is lost in HIV-infected individuals. Coordination was not restored even in those patients who responded to HAART with undetectable viral loads.

***Increase in serum levels of free IL-18 in HIV-infected individuals.*** Decreased levels of IL-18BP with concomitant increased levels of IL-18 resulted in a 3- to 4-fold increase in ratios of IL-18 and IL-18BP concentrations in serum samples obtained from the infected subjects, compared with those in samples obtained from control subjects ($P < .001$) (Figure 1*H*). We calculated free IL-1 concentrations in the serum samples obtained from our study participants, using the law of mass action as described in Materials and Methods. As shown in Figure 1*I*, we found, as was expected, an increase in serum concentrations of free IL-18 in HIV-infected subjects, compared with those in healthy subjects ($P < .001$). Consequently, the difference in free IL-18 concentrations between HIV-infected and healthy donors is more pronounced, compared with the difference in total IL-18 concentrations.

***Effects of HIV infection on production of IL-18 and IL-18BP by human MDMs.*** Macrophages are the main producers of IL-18 and IL-18BP in the body. They are also infected with HIV and act as a viral reservoir in HIV-infected individuals [12]. Therefore, we were interested in determining the effects of HIV-1 infection on the production of IL-18 and its antag-

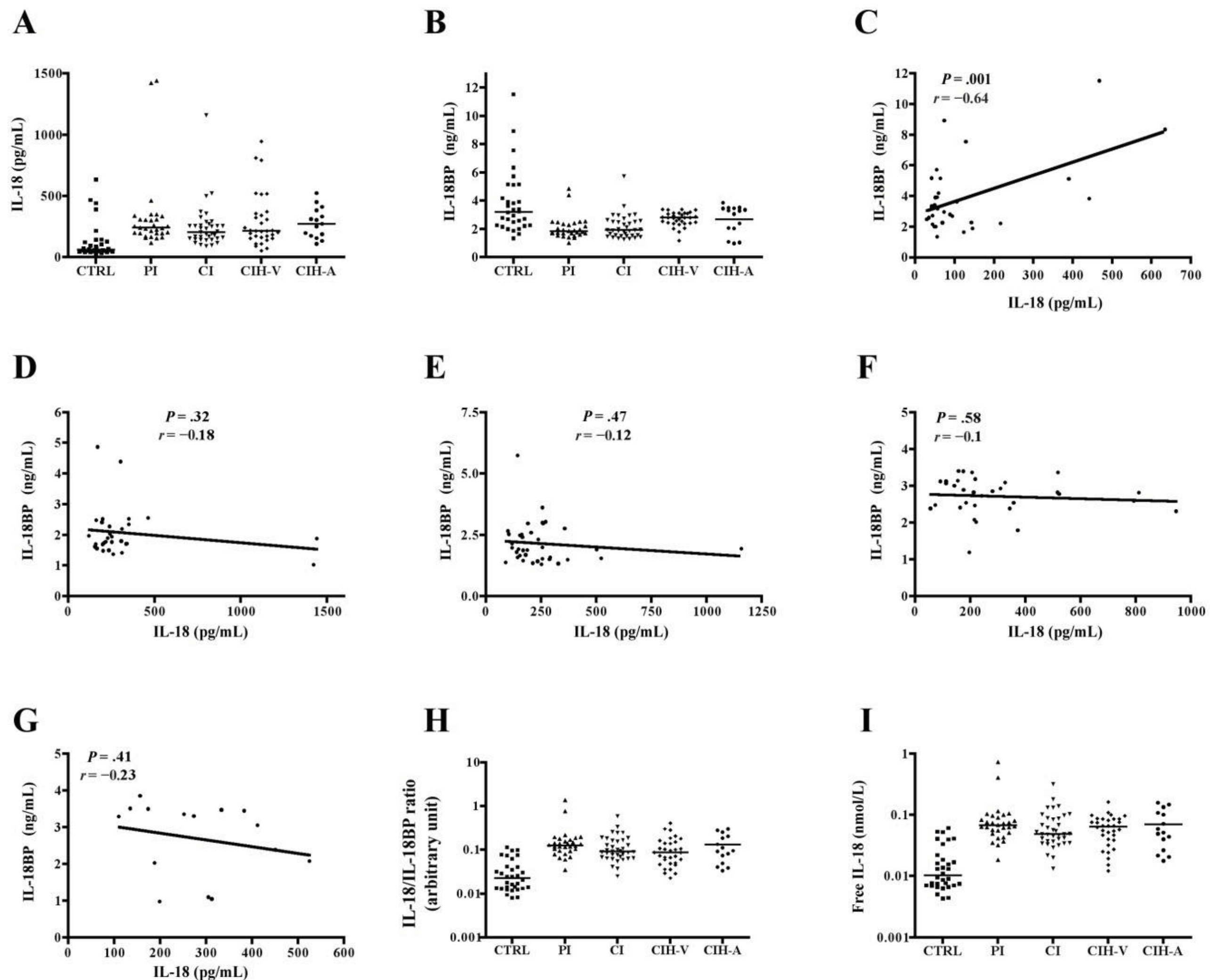


**Figure 1.** Imbalance of interleukin (IL)–18 and IL-18–binding protein (IL-18BP) in human immunodeficiency virus (HIV)–infected patients. IL-18 and IL-18BP concentrations in serum samples were determined by use of a commercial enzyme-linked immunosorbent assay (ELISA) kit. Concentrations of IL-18 *(A)* and IL-18BP *(B)* in the serum samples of 32 control (CTRL) subjects, 30 HIV-infected patients with primary infection (PI), 37 patients with chronic HIV infection (CI), 32 chronically infected patients with viremia who were receiving highly active antiretroviral therapy (HAART) (CIH-V patients), and 15 aviremic patients with CI who were receiving HAART (CIH-A patients). Each dot denotes an individual serum concentration, and the horizontal line in each column denotes the median value for the group. The IL-18 concentrations differed significantly between the CTRL subjects and each group of infected donors ($P < .001$). The same was true for IL-18BP, except between CIH patients (both viremic and aviremic) and CTRL subjects ($P > .05$). *C,* Pearson correlation between IL-18 and IL-18BP concentrations in serum samples obtained from healthy persons. The 2 parameters showed a statistically significant correlation ($P = .001$; $r = 0.64$). *D–G,* Pearson correlation between IL-18 and IL-18BP concentrations in serum samples obtained from HIV-infected patients. The 2 parameters did not show a statistically significant correlation for all groups of patients ($P = .32$ and $r = -0.18$, for patients with PI *[D];* $P = 0.47$ and $r = -0.12$, for patients with CI *[E];* $P = .58$ and $r = -0.1$, for CIH-V patients *[F];* and $P = .41$ and $r = -0.23$, for CIH-A patients *[G]*). *H,* Ratios of IL-18 to IL-18BP in serum samples obtained from the healthy (CTRL) persons and different HIV-infected patients. A dot denotes an IL-18:IL-18BP ratio for an individual serum sample, and the horizontal line in each column denotes the median value for the group. The median values of the ratios differed significantly between the CTRL subjects and each patient group ($P < .001$). *I,* Concentrations of free IL-18 (expressed as nanomoles) in serum samples obtained from healthy (CTRL) persons and HIV-infected patients were calculated using the law of mass action described in Materials and Methods. A dot denotes an individual's concentration of free IL-18, and the horizontal line in each column denotes the median value for the group. Median values differed significantly between the CTRL subjects and each patient group ($P < .001$).

onist by these cells. For this purpose, we infected MDMs with a dual-tropic HIV-1 strain (89.6) that is known to productively infect MDMs [13], and we analyzed the cell culture supernatants for IL-18 and IL-18BP contents. As shown in Figure 2*A*, at each tested time point until 24 days after infection, the virus-infected MDMs produced significantly more ($P < .01$) IL-18, compared with mock-infected MDMs. However, they produced significantly less IL-18BP ($P < .01$) at these time points (Figure

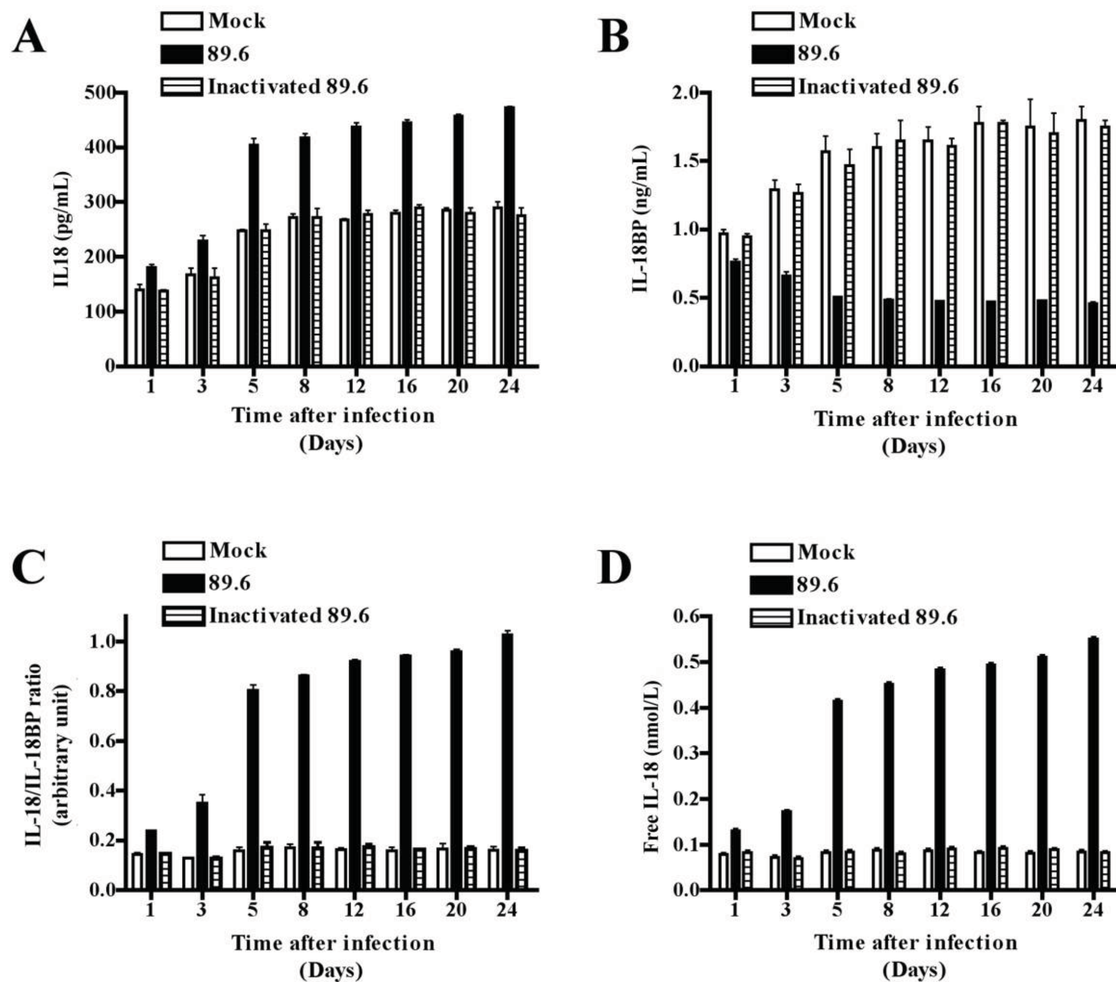


**Figure 2.** Effects of in vitro infection with a dual-tropic human immunodeficiency virus type 1 (HIV-1) strain on production of interleukin (IL)–18 and IL-18–binding protein (IL-18BP) by human monocyte-derived macrophages (MDMs) The MDMs were generated in vitro and infected with a dual-tropic HIV-1 strain (89.6) or with the viral preparation that has been heated at 56°C for 1 h. The concentrations of IL-18 and IL-18BP secreted by these cells were measured in the culture supernatants collected at the indicated time points after infection, by use of enzyme-linked immunosorbent assay kits (*A* and *B,* respectively). The IL-18:IL-18BP ratios *(C)* and the concentrations of free IL-18 *(D)* in the culture supernatants are shown. Essentially similar results were obtained with the MDMs prepared from 3 healthy donors.

2*B*). The ratios between the IL-18 and IL-18BP concentrations present in the supernatants of the mock- and HIV-infected MDMs are shown in Figure 2*C*. As expected, the average ratio was increased for HIV-infected MDMs, compared with mock-infected cells, at each time point.

We further calculated the concentrations of free IL-18 present in these cell culture supernatants and observed an increase in concentrations of free IL-18 in HIV-infected cells, compared with those in mock-infected cells, at each time point (Figure 2*D*). These data suggest that the viral infection itself induces imbalanced production of IL-18 and IL-18BP by MDMs. No significant effects ($P > .05$) on these 2 soluble mediators were observed when the cells were infected with the viral preparation that had been heated at 56°C for 1 h (data not shown). Essentially, similar results were observed when MDMs were infected with an M-tropic viral strain YU-2 (Figure 3). In our hands, both 89.6 and YU-2 replicate in MDMs with similar kinetics, and they reach peak production of progeny viruses by day 8 after infection (data not shown).

Next, we sought to investigate whether HIV-1 replication was required for the observed alterations in IL-18 and IL-18BP production. For this purpose, we infected MDMs with HIV (89.6) for different lengths of time with or without 3TC, a nucleoside reverse-transcriptase inhibitor that blocks viral replication [14]. As shown in Figure 4*A*, treatment with 3TC decreased IL-18 production in cell culture supernatants to levels that were almost equal to those seen in mock-infected cells. The treatment also restored IL-18BP production by MDMs

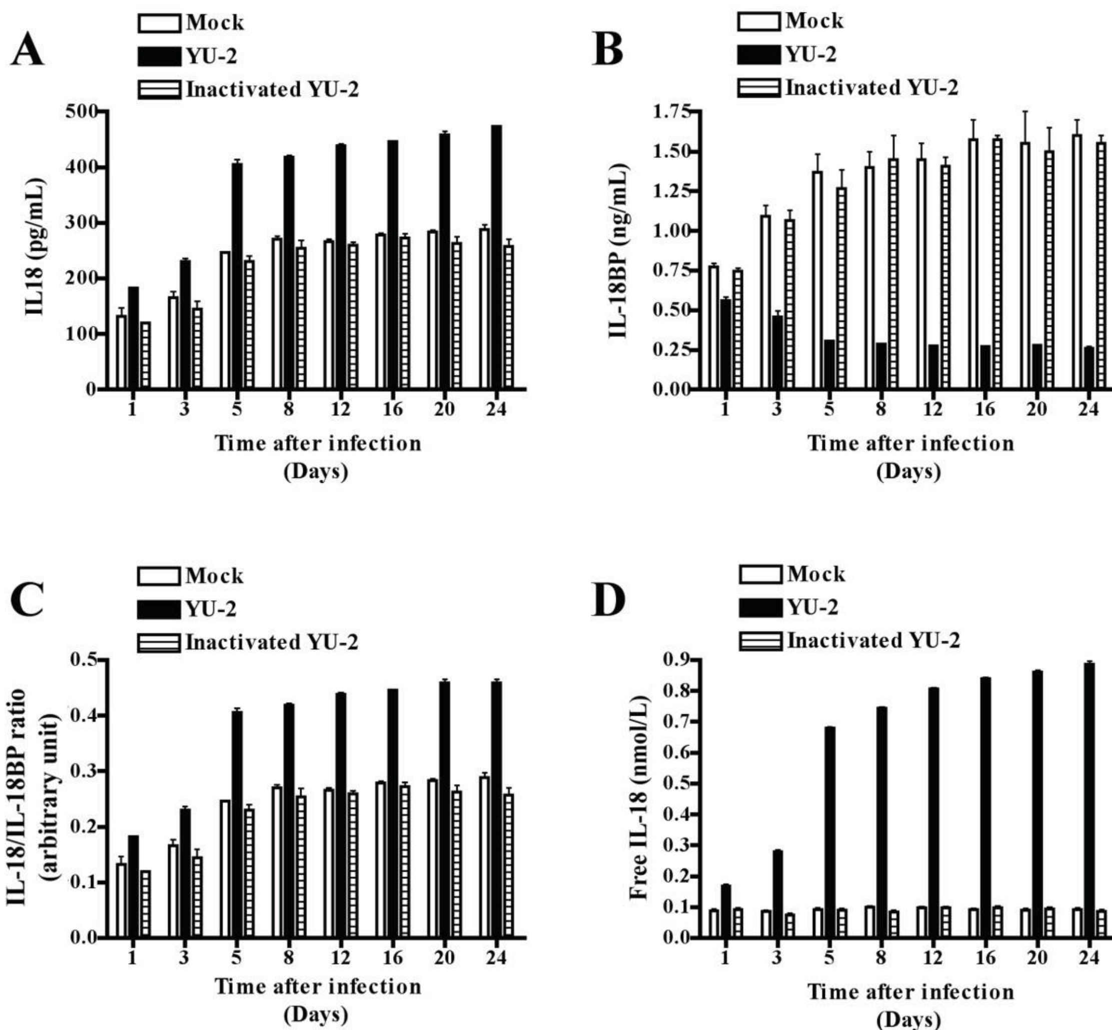


**Figure 3.** Effects of in vitro infection with an M-tropic strain of human immunodeficiency virus type 1 (HIV-1) on production of interleukin (IL)–18 and IL-18–binding protein (IL-18BP) by human monocyte-derived macrophages (MDMs). The MDMs were generated in vitro and infected with an M-tropic HIV-1 strain (YU-2) or with a viral preparation that had been heated at 56°C for 1 h. The concentrations of IL-18 and IL-18BP secreted by these cells were measured in the culture supernatants collected at the indicated time points after infection by use of enzyme-linked immunosorbent assay kits (*A* and *B,* respectively). The IL-18:IL-18BP ratios *(C)* and the concentrations of free IL-18 *(D)* in the culture supernatants are shown. Essentially similar results were obtained with the MDMs prepared from 3 healthy donors.

(Figure 4*B*). Taken together, these data suggest that HIV-1 replication—not binding of the virus to MDMs—results in imbalanced production of IL-18 and IL-18BP by these cells. Essentially similar results were observed when human MDMs were infected with the M-tropic HIV-1 strain YU-2 (data not shown).

***Effect of IL-10 and TGF-β on IL-18BP production by MDMs.*** IL-10 and TGF-$\beta$ are 2 cytokines whose concentrations are often increased in the circulation of HIV-infected patients with AIDS [15, 16]. Therefore, we sought to determine their effects on the production of IL-18BP by human MDMs. For this purpose, we infected MDMs with the dual-tropic virus and added equimolar concentrations of the 2 cytokines for different lengths of time, and we then measured, by use of ELISA, the levels of IL-18BP secreted in the cell culture supernatants. As shown in Figure 5, the infection decreased IL-18BP in the culture supernatants when they were analyzed on days 1, 3, 5, and 8 after infection. Furthermore, the addition of IL-10, IL-18, or TGF-$\beta$ to the MDM cultures also decreased production of this mediator on days 3, 5 and 8 after infection. Of interest, at each of these time points, the effects of the infection and the cytokines on the production of IL-18BP from MDMs were additive (Figure 5). Furthermore, the cytokines also acted in an additive manner in decreasing production of IL-18BP. Taken together, these data suggest that decreased concentrations of circulating IL-18BP from macrophages may oc-

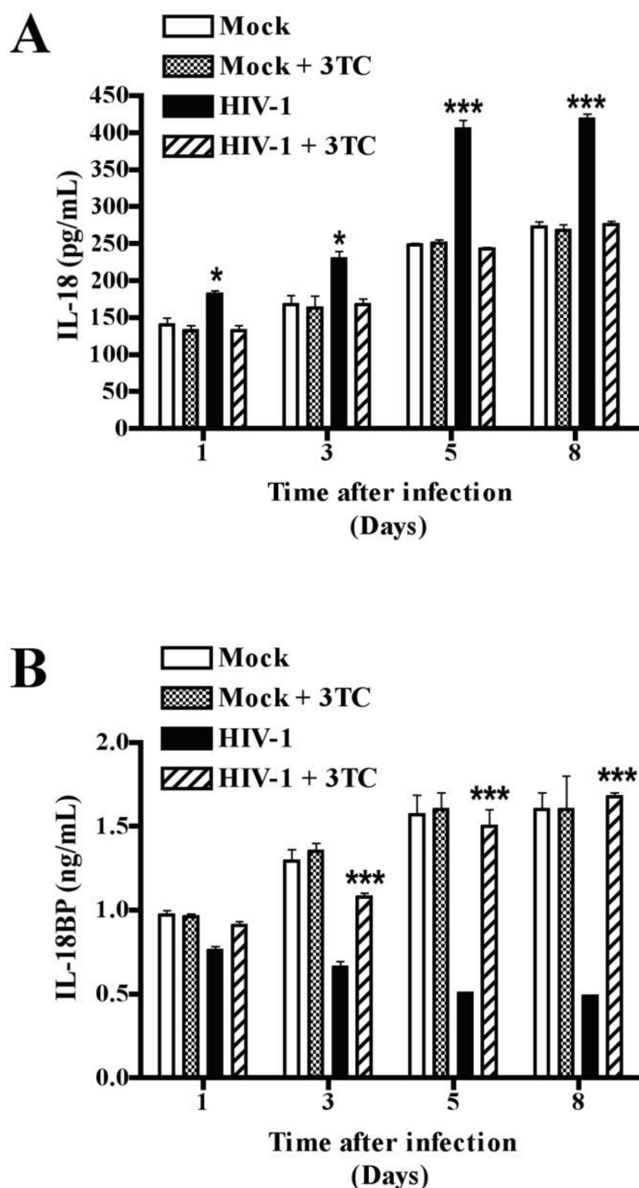


**Figure 4.** Effects of inhibiting human immunodeficiency virus type 1 (HIV-1) replication on production of interleukin (IL)–18 and IL-18–binding protein (IL-18BP) by human monocyte-derived macrophages (MDMs). The MDMs were generated in vitro, infected with a dual-tropic HIV-1 strain (89.6), and treated with or without lamivudine (3TC; 200 nmol/L) for different lengths of time. The concentrations of IL-18 and IL-18BP secreted by these cells were measured in the culture supernatants collected at the indicated time points after infection, by use of enzyme-linked immunosorbent assay kits (*A* and *B,* respectively). Similar results were obtained with the MDMs prepared from 3 healthy donors. One star denotes $P < .05$, and 3 stars denote $P < .001$.

cur in HIV-infected individuals because of their cytokine milieu, even in aviremic conditions. In contrast to their effects on IL-18BP production, cytokine treatments of the MDMs did not significantly ($P > .05$) affect IL-18 production by these cells (data not shown).

In separate experiments, we generated MDMs from 3 viremic patients and 3 HIV-seronegative control subjects. After 8 days of culture at 37°C, no significant difference was observed concerning the concentrations of IL-18 and IL-18BP in their culture supernatants (data not shown).

***Enhancement of HIV-1 replication by IL-18 in human CD4⁺ T cells.*** In accordance with our earlier results [6], no significant correlation existed between serum IL-18 concentrations and viral loads in HIV-infected persons (data not shown). Nevertheless, we were interested in determining the effect of IL-18 on HIV replication, because any replication-enhancing effects of the cytokine may have been suppressed by HAART in the HIV-infected individuals. For this purpose, we infected purified human $CD4^+$ T cells with HIV-1 (strain NL4.3) and added equimolar concentrations of different cytokines (IL-18, IL-12, IL-10, or TGF-$\beta$) alone as well as in different combinations. The supernatants from these cultures were quantified for p24 contents on days 3 and 5 after infection. As shown in Figure 6, we observed a significant ($P < .01$) enhancing effect of recombinant human IL-10, IL-12, and IL-18 on HIV-1 replication. However, no significant ($P \geqslant .05$) effect of TGF-$\beta$ on viral replication was observed. Furthermore, the cytokines did not show any additive effect when they were added together. We also performed experiments to determine the effect of IL-18 and other cytokines on viral replication in MDMs after infecting them with the dual-tropic HIV-1 strain (89.6). IL-18 alone or in combination with other cytokines caused no significant effect on HIV-1 replication in MDMs (data not shown).

## DISCUSSION

The results presented in this study confirm earlier reports from this and other laboratories ([6–8]; reviewed in [9]) regarding increased serum levels of IL-18 in HIV-infected subjects, compared with healthy subjects. Of interest, HAART did not result in normalization of these levels, even in patients who responded to the treatment and had undetectable plasma viral loads. Recent studies have shown that certain anti–HIV-1 drugs (eg, protease inhibitors) induce secretion of proinflammatory cytokines like IL-1$\beta$, tumor necrosis factor–$\alpha$, and IL-6 [17]. Because IL-1$\beta$ and IL-18 belong to the same family and share similar mechanisms of production, it is highly likely that these anti–HIV-1 drugs also induce production of IL-18 by human macrophages.

To our knowledge, the present study is the first to show that, concomitant with an increase in IL-18 concentrations, IL-18BP concentrations are significantly reduced in the circulation in HIV-infected individuals. A practical consequence of the decrease in levels of this IL-18 antagonist is that the concentrations of biologically active IL-18 become even more accentuated in the circulation of these individuals. This would have resulted in increased biological activities of IL-18 in the circulation of

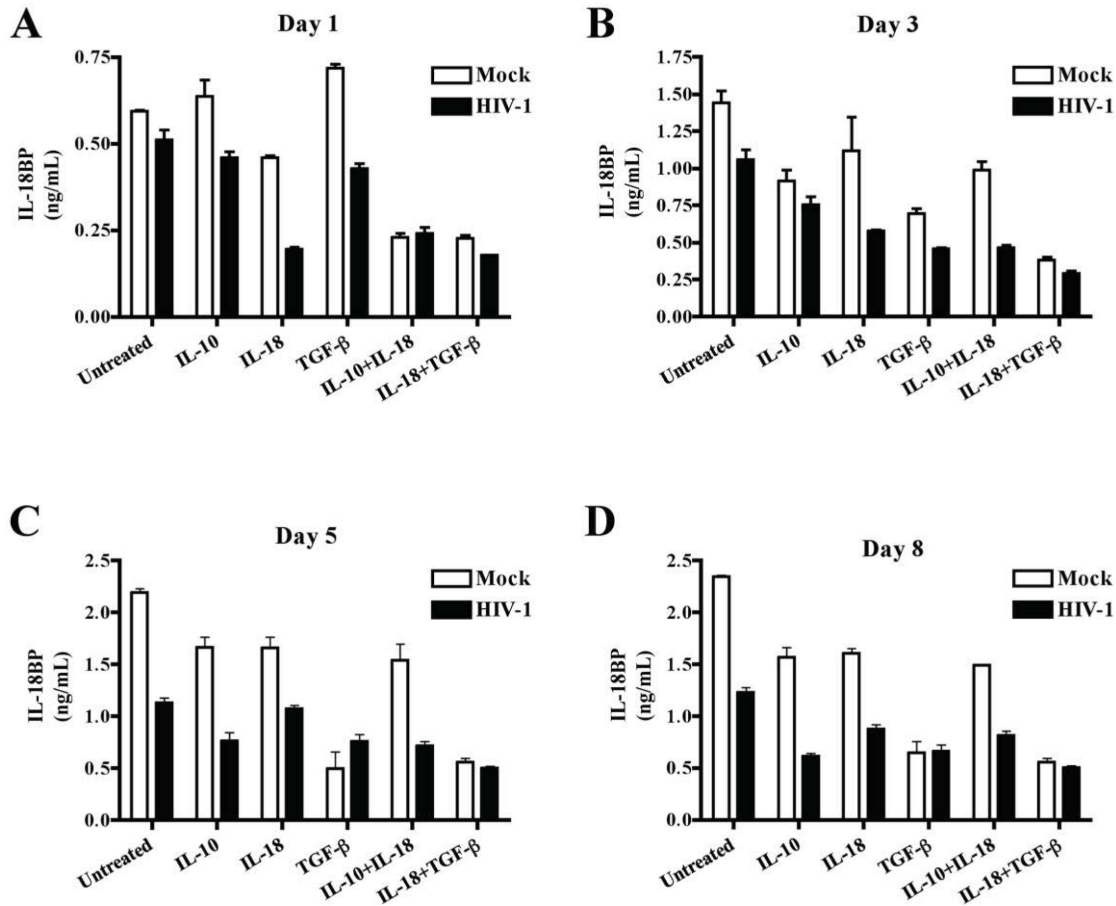


**Figure 5.** Effects of different cytokines on production of interleukin (IL)–18–binding protein (IL-18BP) by human monocyte-derived macrophages (MDMs) with or without infection with human immunodeficiency virus type 1 (HIV-1). The MDMs were generated in vitro and were infected with HIV-1 strain 89.6 with and without equimolar concentrations (15 $\mu$mol/L) of the indicated cytokines or their combinations. At the indicated time points after infection, the culture supernatants were collected and analyzed for their IL-18BP contents. Note the differential effects of certain cytokines (eg, IL-18) on the infected and mock-infected cells, as well as the additive effects of certain cytokine combinations (eg, IL-18 and transforming growth factor [TGF]–$\beta$), irrespective of infection status, on IL-18BP production by the MDMs. Similar results were obtained with the MDMs prepared from 3 healthy donors.

HIV-infected persons. Indeed, we have previously demonstrated that the biological activity of IL-18 is increased in serum samples obtained from HIV-infected individuals [6]. In this study, we also provide experimental evidence to show that infection of macrophages with HIV-1 contributes to imbalanced production of IL-18 and its antagonist in HIV-infected individuals. It is noteworthy that in vivo infection of macrophages in many tissues of the body has been demonstrated in HIV-infected persons [12]. It is widely believed that macrophages are the first type of cells to be infected via a mucosal route (which is the predominant route of infection in humans). These cells also represent an important reservoir of infection in HAART-treated HIV-infected persons [12]. These observations imply that the observed imbalance in levels of IL-18 and IL-18BP may occur in early stages of the infection. Indeed, increased concentrations of circulating IL-18 have been reported in early stages of the infection [18].

Studies of IL-18BP have shown that its production is induced in the body as a negative-feedback mechanism in response to IL-18 for controlling proinflammatory biological effects of the cytokine. This induction occurs, at least in part, as a result of IL-18–mediated IFN-$\gamma$ production by NK and T cells. It is noteworthy that IFN-$\gamma$ is a powerful stimulus for production of IL-18BP [19]. The HIV-induced decrease in production of this soluble mediator seems to occur independently of IFN-$\gamma$, because MDMs are not known to produce this cytokine. Furthermore, our data show that IL-18 can also directly inhibit production of its antagonist in human MDMs. Moreover, decreased production of IFN-$\gamma$ in HIV-infected persons has been reported elsewhere [20]. Decreased production of TH1 type cytokines, of which IFN-$\gamma$ is the representative one, has been implicated in the pathogenesis of AIDS [21]. Although IL-18 is a powerful inducer of IFN-$\gamma$ in NK and T cells, it does so only in combination with other cytokines (eg, IL-12, IL-21,

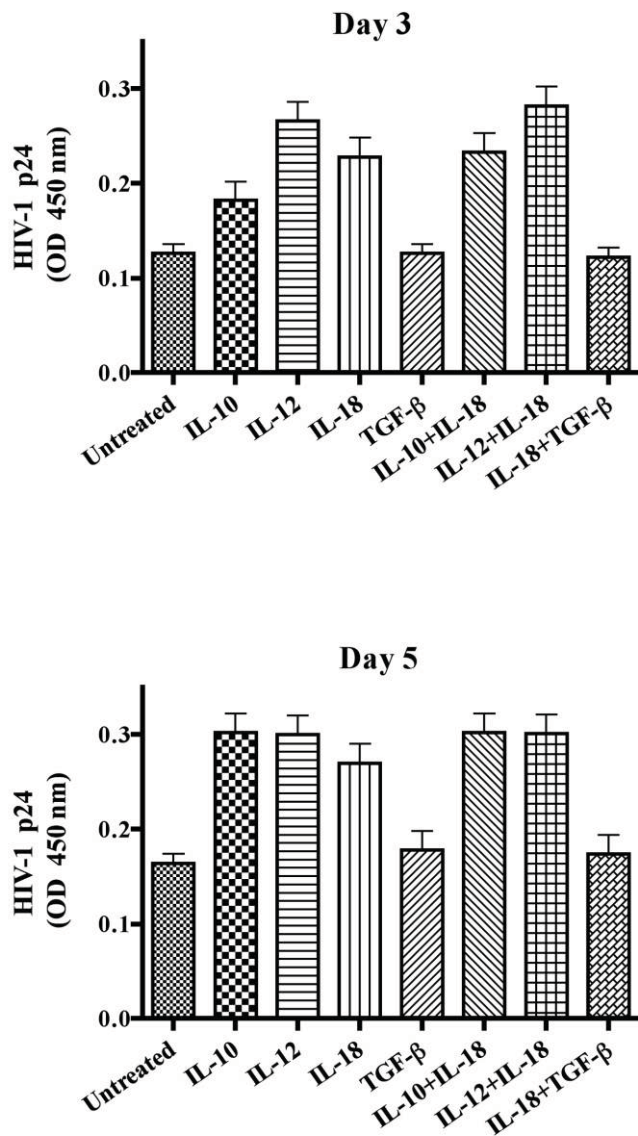


**Figure 6.** Effects of interleukin (IL)–18, alone and in combination with other cytokines, on production of HIV-1 in human CD4+ T cells. Human CD4+ T cells from uninfected donors were purified from activated peripheral blood mononuclear cells (phytohemagglutinin and interleukin-2), infected in vitro with a T-tropic human immunodeficiency virus type 1 (HIV-1) strain (NL4.3) and treated with equimolar concentrations (15 $\mu$mol/L) of the indicated cytokines, either alone or in combinations, for the indicated lengths of time. The HIV-1 p24 level was measured in the culture supernatants by use of an enzyme-linked immunosorbent assay kit. Similar results were obtained for 3 different donors. OD, optical density; TGF-$\beta$, transforming growth factor–$\beta$.

etc). IL-18 alone induces little of this interferon. Because production of IL-12, IL-15, and IL-21 markedly decreases in HIV-infected persons [22–24], it is not surprising that production of IFN-$\gamma$ also decreases in these patients. Furthermore, immunosuppressive cytokines, such as TGF-$\beta$ and IL-10, also inhibit production of this interferon in response to IL-18 and other stimuli. The concentrations of these immunosuppressive cytokines are increased in HIV-infected persons [6, 23, 25–27]. We have shown here that treatment of macrophages with these cytokines decreases production of IL-18BP, apparently in an IFN-$\gamma$–independent manner. Thus, the cytokine milieu prevalent in the circulation of HIV-infected persons also contributes to decreased production of IL-18BP in these patients.

Conflicting results concerning the effects of IL-18 on HIV-1 replication have been published in the literature [9]. The cytokine stimulates HIV-1 replication in vitro in chronically infected human monocytic and T cell lines but not in primary human cells [28, 29]. However, it was reported to inhibit replication of both M- and T-tropic HIV-1 strains in human PBMCs, and the inhibitory effect was ascribed to IL-18–induced IFN-$\gamma$ production [30]. In PBMC cultures, the inhibitory effects of IL-18 may prevail because of the cytokine-induced production of IFN-$\gamma$ by NK cells. However, IL-18 promotes HIV-1 replication in isolated CD4+ T cells, chronically infected T cell lines, and HIV-infected monocytic cells in which IL-18 alone would induce little IFN-$\gamma$. As mentioned earlier, IL-18 alone induces little IFN-$\gamma$. It induces this interferon only when acting in collaboration with other cytokines (eg, IL-12). Production of the collaborating cytokines becomes compromised in HIV-infected individuals, often in early stages of the infection (as reviewed in [23]). Furthermore, increased concentrations of immunosuppressive cytokines (ie, TGF-$\beta$ and IL-10) in the circulation of HIV-infected individuals would also inhibit production of IFN-$\gamma$ by their PBMCs. Under these circumstances, it should not be surprising if IL-18 promotes HIV-1 replication in the PBMCs (and tissues) of HIV-infected persons. This finding may explain the reported association of AIDS progression with increased serum levels of the cytokine in HIV-infected subjects [7, 31, 32].

In short, the present study provides novel insights concerning the regulation of IL-18 and its antagonist in HIV-1 infection.

## Acknowledgment

We thank Mario Legault for administrative coordination of collection of blood specimens from human immunodeficiency virus–infected patients.

## References

1. Dinarello C. IL-18: a TH1-inducing, proinflammatory cytokine and new member of the IL-1 family. J Allergy Clin Immunol **1999**; 103: 11–24.
2. Dinarello C. Interleukin-18 and the pathogenesis of inflammatory diseases. Semin Nephrol **2007**; 27:98–114.
3. Kim S-H, Eisenstein M, Reznikov L, et al. Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. Proc Natl Acad Sci **2000**; 97:1190–5.
4. Kumar S, McDonnell PC, Lehr R, et al. Identification and initial characterization of four novel members of the interleukin-1 family. J Biol Chem **2000**; 275:10,308–14.

5. Novick D, Kim S-H, Fantuzzi G, Reznikov LL, Dinarello CA, Rubinstein M. Interleukin-18 binding protein: a novel modulator of the Th1 cytokine response. Immunity **1999**; 10:127–36.
6. Ahmad R, Sindhu STA, Toma E, Morisset R, Ahmad A. Elevated levels of circulating interleukin-18 in human immunodeficiency virus-infected individuals: role of peripheral blood mononuclear cells and implications for AIDS pathogenesis. J Virol **2002**; 76:12,448–56.
7. Song W, Wilson CM, Allen S, et al. Interleukin 18 and human immunodeficiency virus type I infection in adolescents and adults. Clin Exp Immunol **2006**; 144:117–24.
8. Torre D, Speranza F, Martegani R, et al. Circulating levels of IL-18 in adult and paediatric patients with HIV-1 infection. AIDS **2000**; 14: 2211–2
9. Torre D, Pugliese A. Interleukin-18: a proinflammatory cytokine in HIV-1 infection. Curr HIV Res **2006**; 4:423–30.
10. Ndongala M, Peretz Y, Boulet S, et al. HIV Gag p24 specific responses secreting IFN-$\gamma$ and/or IL-2 in treatment-naïve individuals in acute infection early disease (AIED) are associated with low viral load. Clin Immunol **2009**; 131:277–87.
11. Mazodier K, Marin V, Novick D, et al. Severe imbalance of IL-18/IL-18BP in patients with secondary hemophagocytic syndrome. Blood **2005**; 106:3483–9.
12. Aquaro S, Caliò R, Balzarini J, Bellocchi MC, Garaci E, Perno CF. Macrophages and HIV infection: therapeutical approaches toward this strategic virus reservoir. Antiviral Res **2002**; 55:209–25.
13. Yi Y, Isaacs SN, Williams DA, et al. Role of CXCR4 in cell-cell fusion and infection of monocyte-derived macrophages by primary human immunodeficiency virus type 1 (HIV-1) strains: two distinct mechanisms of HIV-1 dual tropism. J Virol **1999**; 73:7117–25.
14. Coates JA, Cammack N, Jenkinson HJ, et al. (-)-2'-deoxy-3'-thiacytidine is a potent, highly selective inhibitor of human immunodeficiency virus type 1 and type 2 replication in vitro. Antimicrob Agents Chemother **1992**; 36:733–9.
15. Stylianou E, Aukrust P, Kvale D, Müller F, Frøland S. IL-10 in HIV infection: increasing serum IL-10 levels with disease progression—down-regulatory effect of potent anti-retroviral therapy. Clin Exp Immunol **1999**; 116:115–20.
16. Wiercińska-Drapalo A, Flisiak R, Jaroszewicz J, Prokopowicz D. Increased plasma transforming growth factor-$\beta$1 is associated with disease progression in HIV-1-infected patients. Viral Immunol **2004**; 17: 109–13.
17. Chen L, Jarujaron S, Wu X, et al. HIV protease inhibitor lopinavir-induced TNF-$\alpha$ and IL-6 expression is coupled to the unfolded protein response and ERK signaling pathways in macrophages. Biochem Pharmacol **2009**; 78:70–7.
18. Sailer CA, Pott GB, Dinarello CA, et al. Whole-blood interleukin-18 level during early HIV-1 infection is associated with reduced CXCR4 coreceptor expression and interferon-$\gamma$ levels. J Infect Dis **2007**; 195: 734–8.
19. Hurgin V, Novick D, Rubinstein M. The promoter of IL-18 binding protein: activation by an IFN-$\gamma$-induced complex of IFN regulatory factor 1 and CCAAT/enhancer binding protein beta. Proc Natl Acad Sci USA **2002**; 99:16,957–62.
20. Koirala J, Adamski A, Koch L, et al. Interferon-gamma receptors in HIV-1 infection. AIDS Res Hum Retroviruses **2008**; 24:1097–102.
21. Clerici M, Shearer G. The Th1-Th2 hypothesis of HIV infection: new insights. Immunol Today **1994**; 15:575–81.
22. Ahmad A, Ahmad R, Toma E, Morisset R, Menezes J. Impaired induction of IL-15 in response to herpes simplex virus type 1 infection in peripheral blood mononuclear cells of HIV-infected patients. AIDS **2000**; 14:744–6.
23. Iannello A, Debbeche O, Samarani S, Ahmad A. Antiviral NK cell responses in HIV infection: II. Viral strategies for evasion and lessons for immunotherapy and vaccination. J Leukoc Biol **2008**; 84:27–49.
24. Iannello A, Tremblay C, Routy J, Boulassel M, Toma E, Ahmad A. Decreased levels of circulating IL-21 in HIV-infected AIDS patients: correlation with $CD4^+$ T cell counts. Viral Immunol **2008**; 21:385–8.
25. Allen J, Wong H, Guyre P, Simon G, Wahl S. Association of circulating receptor Fc gamma RIII-positive monocytes in AIDS patients with elevated levels of transforming growth factor-beta. J Clin Invest **1991**; 87:1773–9.
26. Lotz M, Seth P. TGF$\beta$ and HIV infection. Ann N Y Acad Sci **1993**; 685:501–11.
27. Parato K, Kumar A, Badley A, et al. Normalization of natural killer cell function and phenotype with effective anti-HIV therapy and the role of IL-10. AIDS **2002**; 16:1251–6.
28. Klein S, Klebba C, Kauschat D, et al. Interleukin-18 stimulates HIV-1 replication in a T-cell line. Eur Cytokine Netw **2000**; 11:47–52.
29. Pugliese A, Gennero L, Vidotto V, Speranza F, Tambini R, Torre D. Interleukin-18 enhances HIV-1 production in a human chronically-infected T cell line (H9-V). Cell Biochem Func **2002**; 20:333–7.
30. Choi HJ, Dinarello CA, Shapiro L. Interleukin-18 inhibits human immunodeficiency virus type 1 production in peripheral blood mononuclear cells. J Infect Dis **2001**; 184:560–8.
31. Wiercinska-Drapalo A, Jaroszewicz J, Flisiak R, Prokopowicz D. Plasma interleukin-18 is associated with viral load and disease progression in HIV-1-infected patients. Microbes Infect **2004**; 6:1273–7.
32. Stylianou E, Bjerkeli V, Yndestad A, et al. Raised serum levels of interleukin-18 is associated with disease progression and may contribute to virological treatment failure in HIV-1-infected patients. Clin Exp Immunol **2003**; 132:462–6.

## 12- Résultats concernant les études sur l'IL-21

### 12-1 Régulation de l'IL-21 lors de l'infection par le VIH-1

Les résultats obtenus lors de cette étude concernant la dérégulation de la production d'IL-21 chez les patients infectés par le VIH-1 ainsi que les conséquences sur le système immunitaire sont présentés sous le format de deux articles de recherche. Ces articles ont été soumis à un processus de révision par les pairs avant leur publication.

**Article 8 : Iannello A**, Tremblay C, Routy JP, Boulassel MR, Toma E and Ahmad A. Decreased levels of circulating IL-21 in HIV-infected AIDS patients: Correlation with HIV CD4$^{+}$ T cell counts. 2008. ***Viral Immunology* 21(3): 385-388**.

**Article 9 : Iannello A**, Boulassel MR, Samarani S, Debbeche O, Tremblay C, Toma E, Routy JP and Ahmad A. Dynamics and consequences of IL-21 production in HIV-infected persons: A longitudinal and cross sectional study. 2010. ***Journal of Immunology* 184(1): 114-26**.

**Résumé :** L'IL-21 joue un rôle clef dans le contrôle des infections virales chroniques. Nous avons déterminé la dynamique de la production d'IL-21 lors de l'infection par le VIH-1 et sa conséquence sur la survie des cellules T CD4$^{+}$ et la fréquence des cellules T CD8$^{+}$ spécifiques au VIH-1. Nous avons démontré que sa production est compromise tôt au cours de l'infection et que les concentrations d'IL-21 corrélent avec le compte de cellules T CD4$^{+}$ chez les personnes infectées. Notre étude a démontré que le traitement antirétroviral restaure partiellement la production de l'IL-21. De plus, l'infection par le VIH-1 des cellules T CD4$^{+}$ humaines inhibe sa production en réduisant l'expression du facteur de transcription c-Maf. Nous avons démontré que la fréquence des cellules T CD4$^{+}$ spécifiques au VIH-1 qui produisent de l'IL-21 est réduite chez les patients virémiques. Nous démontrons aussi que l'IL-21 recombinante empêche l'apoptose spontanée des cellules T CD4$^{+}$ de patients infectés et que l'absence d'IL-21 réduit la fréquence des cellules T CD8$^{+}$ spécifiques au VIH-1.

# Article 8

**Iannello A**, Tremblay C, Routy JP, Boulassel MR, Toma E and Ahmad A. Decreased levels of circulating IL-21 in HIV-infected AIDS patients: Correlation with HIV $CD4^+$ T cell counts. 2008. ***Viral Immunology*** **21(3): 385-388**.

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# Decreased Levels of Circulating IL-21 in HIV-Infected AIDS Patients: Correlation with CD4$^+$ T-Cell Counts

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## Abstract

IL-21 is a relatively newly discovered multifunctional and pleiotropic cytokine. It is produced primarily by CD4$^+$ T cells, the principal targets of the virus, and therefore this cytokine has special relevance to HIV infection. Here we show for the first time that serum levels of this cytokine are significantly reduced in HIV-infected AIDS patients and correlate significantly with their CD4$^+$ T-cell counts. These data suggest that the cytokine levels could act as a valuable biomarker for the progression of AIDS.

## Introduction

Interleukin-21 (IL-21) is a relatively recently discovered multifunctional and pleiotropic cytokine (9,11). It is a member of the common $\gamma$-chain-using cytokine family that also includes IL-2, IL-4, IL-7, IL-9, and IL-15. The cytokine has been shown to exert significant immune enhancing and regulatory functions (11). It has been demonstrated that CD4$^+$ T cells are the main producers of this cytokine in the human body (9). It is noteworthy that these cells are also the primary targets of HIV in infected persons. They are progressively depleted in HIV-infected persons as the infection progresses to full-blown AIDS. The depletion of CD4$^+$ T cells constitutes an important hallmark of AIDS (8). In this connection IL-21 bears special relevance to HIV infection, and for this reason we were interested in investigating the production of this cytokine in HIV-infected persons.

In order to address this issue, we measured IL-21 in the sera of 27 adult HIV-infected AIDS patients and compared the sera with that from 27 age-matched HIV-seronegative healthy subjects. The blood samples were taken from the study participants after obtaining their written informed consent. All the patients had one or more AIDS-defining conditions and were receiving highly active antiretroviral therapy (HAART) that comprised at least one protease inhibitor (saquinavir, ritonavir, or indinavir) and one or two reverse transcriptase inhibitors (stavudine, lamivudine, zidovudine, or didanosine). The characteristics of the patients are detailed in Table 1. The concentrations of IL-21 were determined in the serum samples using a commercial enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, San Diego, CA) per the manufacturer's recommended protocol. The kit is based on a sandwich ELISA that utilizes two monoclonal antibodies directed against two different epitopes of human IL-21, and could detect levels as low as 31 pg/mL of the cytokine. We used two-tailed Student's unpaired *t*-test for comparing mean concentrations of the cytokine between the infected and control subjects with PRISM software (GraphPad, San Diego, CA).

As shown in Fig. 1A, the mean serum levels of IL-21 are significantly ($p = 0.0087$) reduced in the HIV-infected AIDS patients as compared to healthy subjects. On the average, the patient levels were half of the control group levels (285 ± 25 pg/mL versus 534 ± 77 pg/mL, respectively). Furthermore, using Pearson's correlation with GraphPad PRISM software, we found that the IL-21 concentrations showed a significant positive correlation ($p = 0.0006$; $r = 0.79$) with CD4$^+$ T-cell counts of the patients (Fig. 1B). Nevertheless, no correlation was found between the serum IL-21 levels and the viral load (the number of copies of HIV RNA per milliliter of peripheral blood) of the HIV-infected AIDS patients (data not shown).

To the best of our knowledge, this is the first report describing a decrease in the serum concentrations of IL-21 and their correlation with CD4$^+$ T-cell counts in HIV-infected AIDS patients. Our unpublished data shows that HIV *per se*

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Table 1. Characteristics of the Patients

| *Patients* | *CD4$^{+}$ (cells/μL)* | *Viral load (log 10)* | *Clinical stage* | *Duration* |
|---|---|---|---|---|
| 1 | 299 | 4.54 | C2 | >4 y |
| 2 | 345 | 3.60 | C2 | >3 y |
| 3 | 211 | 2.70 | C2 | ND |
| 4 | 112 | 4.70 | B3 | 4 y 6 mo |
| 5 | 165 | 2.70 | C3 | 5 y 7 mo |
| 6 | 255 | 2.70 | B2 | ND |
| 7 | 280 | 2.70 | C2 | ND |
| 8 | 16 | 4.55 | C3 | >6 y |
| 9 | 463 | 2.70 | C2 | 6 y |
| 10 | 214 | 2.80 | C2 | 3 y |
| 11 | 248 | 4.85 | B2 | ND |
| 12 | 343 | 2.80 | B2 | >3 y |
| 13 | 473 | 2.70 | C2 | 10 y |
| 14 | 271 | 2.70 | B2 | 8 y 8 mo |
| 15 | 72 | 3.56 | C3 | ND |
| 16 | 211 | 2.70 | C2 | ND |
| 17 | 154 | 3.56 | C3 | 8 y 2 mo |
| 18 | 180 | 4.84 | A3 | 3 y |
| 19 | 124 | 3.20 | C2 | >3 y |
| 20 | 746 | 2.70 | C1 | 4 y |
| 21 | 126 | 2.80 | C3 | 4 y |
| 22 | 86 | 4.20 | B3 | ND |
| 23 | 177 | 4.30 | C3 | 4 y |
| 24 | 69 | 2.70 | A3 | >3 y |
| 25 | 420 | 2.70 | C2 | 5 y |
| 26 | 171 | 4.79 | C3 | 4 y |
| 27 | 230 | 3.08 | B2 | 3 y 9 mo |
| Mean ± SE | 240 ± 155 | 3.40 ± 0.85 | | |
| Median | 211 | 2.80 | | |
| Range | 16–746 | 2.70–4.85 | | |

The clinical stages are as described in ref. 2a.

induces IL-21 production in human CD4$^{+}$ T cells. Another virus, lymphocytic choriomeningitis virus, which is unrelated to HIV, also induces IL-21 mRNA in human cells (6). Thus HIV itself may be inducing IL-21 production from CD4$^{+}$ T cells. However, these cells are gradually depleted in HIV-infected patients and they represent the main producers of this cytokine, and their depletion results in a decrease in serum IL-21 concentrations in these patients. This is supported by the fact that the extent of the decreased production is dependent upon the degree of depletion of the cells. Although, classical CD4$^{+}$ NKT cells with invariant TCR have also been shown to produce IL-21, these cells constitute a very small proportion of the IL-21–producing cell pool in the body. Interestingly, these cells are also infected with HIV and decrease in numbers in HIV-infected patients (3,13).

In this study we did not find a significant correlation between IL-21 levels and HIV load of the patients. This could be explained by the fact that our study patients were receiving HAART. This antiviral chemotherapy reduces viral load dramatically, but is often not as quick and effective in restoring CD4$^{+}$ T-cell counts. Nevertheless HAART may have affected the production of this cytokine in our HIV-infected patients. In order to investigate the impact of HAART on IL-21 production, we obtained serum samples from 25 age-matched chronically infected patients that were not undergoing HAART or any other antiretroviral therapy and measured the concentrations of the cytokine in their sera. As shown in Fig. 1C, the IL-21 concentrations in these patients were significantly lower compared to those of the control and HAART-treated HIV-infected patients (285 ± 25 pg/mL in HAART-treated patients versus 40 ± 4 pg/mL in infected patients not receiving HAART). The average concentration in the HAART-treated patients was sevenfold higher than that of the untreated HIV-infected individuals. Nevertheless, we found that IL-21 concentrations for the treatment-naïve patients also showed a significant positive correlation with their CD4$^{+}$ T-cell counts (data not shown). These data show that HAART enhances the concentrations of circulating IL-21 in HIV-infected persons.

CD4$^{+}$ T-cell depletion is a hallmark of AIDS. This depletion is believed to be responsible, at least in part, for the immunodeficiency seen in HIV-infected AIDS patients. Decreased production of IL-21 in HIV-infected persons may affect their ability to mount an effective antiviral immune response and control HIV replication. The cytokine promotes proliferation and accumulation of antigen-specific CD8$^{+}$ effector T cells (2). It also increases the survival and cytolytic potential of these cells, especially in synergism with other cytokines (e.g., IL-15 and IL-18) (14,15). Furthermore, the cytokine also acts in synergy with these cytokines to enhance cytolytic potential of NK cells (12). More importantly, IL-21 also induces differentiation of naïve CD4$^{+}$ T cells into Th-17 cells, which play an important role in inducing inflammation and control of invading pathogens (7). It is also well

known that IL-21 has a significant influence on the regulation of B-cell functions *in vivo*: it promotes differentiation of B cells into memory and plasma cells, and suppresses IgE production (4,5). Therefore IL-21 deficiency may be responsible, at least in part, in inducing defective antiviral cellular immunity and enhanced IgE levels, which are seen in HIV-infected AIDS patients.

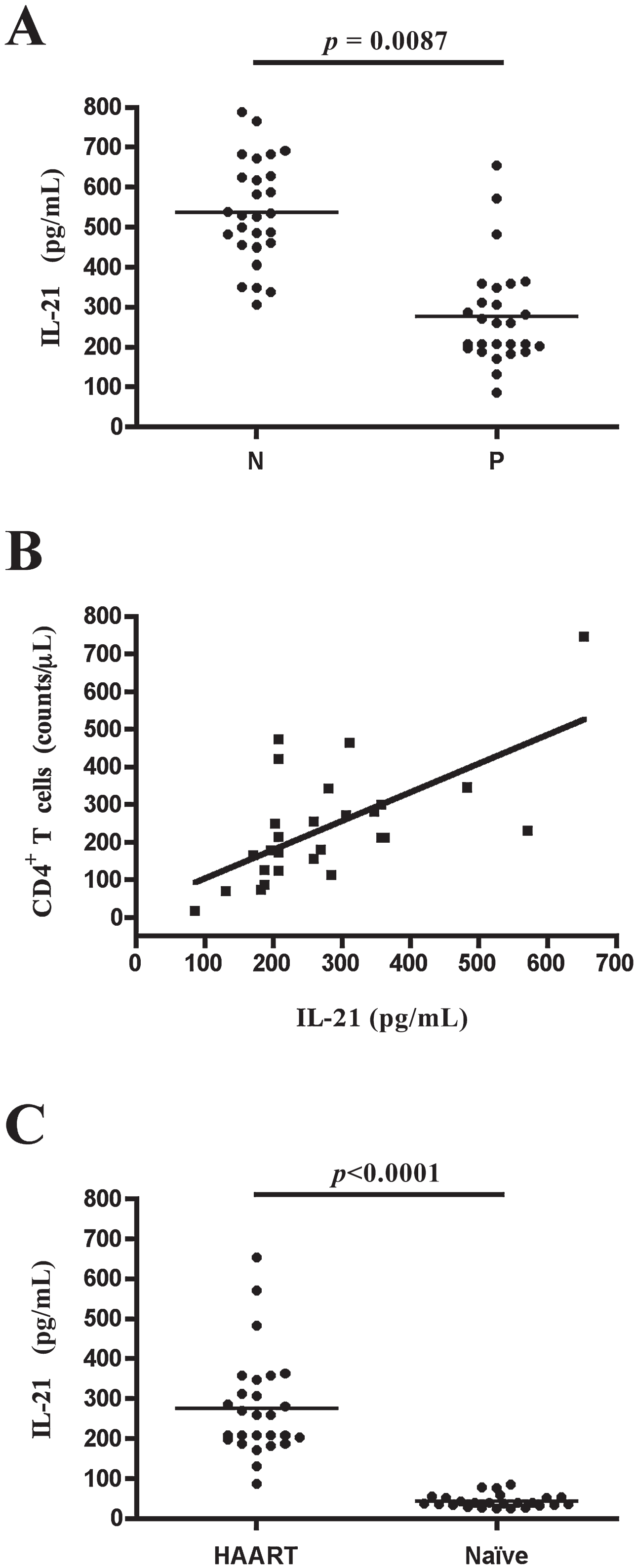


**FIG. 1.** Concentrations of IL-21 in the sera of HIV-infected/AIDS patients. (**A**) IL-21 concentrations were determined in serum samples by using a commercial ELISA kit. The figure depicts the concentrations of IL-21 in the sera from 27 HIV-infected/AIDS patients (P) and an equal number of age-matched HIV-seronegative control subjects (N). A dot indicates an individual serum concentration and the horizontal line in each column indicates the group mean. The average concentrations of the cytokine differed significantly between the two groups of donors ($p = 0.0087$; Student's two-tailed *t*-test). (**B**) Pearson correlation between concentrations of IL-21 in the sera from 27 HIV-infected/AIDS patients and their $CD4^+$ T-cell counts. The two parameters showed a statistically significant correlation ($p = 0.0006$; $r = 0.79$). (**C**) The figure depicts the concentrations of IL-21 in the sera from 27 HIV-infected/AIDS patients receiving HAART and from 25 treatment-naïve HIV-infected patients. A dot indicates an individual serum concentration and the horizontal line in each column indicates the group mean. The average concentrations of the cytokine differed significantly between the two groups of donors ($p < 0.0001$; Student's two-tailed *t*-test).

Our results suggest that serum IL-21 concentrations may be used as a surrogate biomarker for $CD4^+$ T-cell counts and AIDS progression in HIV-infected persons. It may give important information about the $CD4^+$ T-cell counts of the patients, especially in situations in which researchers or clinicians may have access only to serum samples of the patients. The levels may also act as a measure of the patient's response to HAART or any other antiretroviral therapy. A decreased production of IL-21 in these patients also suggests that recombinant human IL-21 may be considered as a therapeutic agent to boost anti-HIV immune responses in HIV-infected AIDS patients. It is noteworthy that a recent study has demonstrated immune-enhancing effects of IL-21 when used as an adjuvant in combination with an HIV *env*-expressing vaccinia virus (1). The recombinant virus induced strong anti-HIV CTL responses and resisted viral challenge in a mouse model when used along with an IL-21-expression plasmid. This study also showed that IL-21 and IL-15 act in synergism to increase antibody-dependent cellular cytotoxicity and complement-dependent lysis of gp120-expressing target cells through augmentation of Env-specific IgG antibody levels (1). Moreover, unlike several other cytokines (e.g., IL-2 and IL-12), IL-21 is less mitogenic, minimally enhances HIV replication, and is relatively safe in humans (our unpublished data; 10).

In conclusion, serum IL-21 levels may serve as an important immune parameter for determining progression of HIV infection and response to antiretroviral therapy in HIV-infected persons.

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## References


1. Bolesta E, Kowalczyk A, Wierzbicki A, et al.: Increased level and longevity of protective immune responses induced by DNA vaccine expressing the HIV-1 Env glycoprotein when combined with IL-21 and IL-15 gene delivery. J Immunol 2006;177:177–191.
2. Casey KA, and Mescher MF: IL-21 promotes differentiation of naive CD8 T cells to a unique effector phenotype. J Immunol 2007;178:7640–7648.

2a. Castro KG, Ward JW, Slusther L, et al.: Revised classification of system on HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMW Rep 1992;41:1–9.

3. Coquet JM, Kyparissoudis K, Pellicci DG, Besra G, Berzins SP, Smyth MJ, and Godfrey DI: IL-21 is produced by NKT cells and modulates NKT cell activation and cytokine production. J Immunol 2007;178:2827–2834.
4. Ettinger R, Sims GP, Fairhurst A-M, et al.: IL-21 induces differentiation of human naive and memory B cells into antibody-secreting plasma cells. J Immunol 2005;175:7867–7879.
5. Harada M, Magara-Koyanagi K, Watarai H, et al.: IL-21-induced B$\varepsilon$ cell apoptosis mediated by natural killer T cells suppresses IgE responses. J Exp Med 2006;203:2929–2937.
6. Holm C, Nyvold CG, Plaudan SR, Thomsen AR, and Hokland M: Interleukin-21 mRNA expression during virus infections. Cytokine 2006;33:41–45.
7. Korn T, Bettelli E, Gao W, et al.: IL-21 initiates an alternative pathway to induce proinflammatory TH17 cells. Nature 2007;448:484–487.
8. Pantaleo G, Graziosi C, and Fauci AS: The role of lymphoid organs in the pathogenesis of HIV infection. Semin Immunol 1993;5:157–163.
9. Parrish-Novak J, Dillon SR, Nelson A, et al.: Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. Nature 2000;408:57–63.
10. Skak K, Kragh M, Hausman D, Smyth MJ, and Sivakumar PV: Interleukin 21: combination strategies for cancer therapy. Nat Rev Drug Discov 2008;7:231–240.
11. Spolski R, and Leonard WJ: Interleukin-21: Basic biology and implications for cancer and autoimmunity. Ann Rev Immunol 2008;26:57–79.
12. Strengell M, Matikainen S, Siren J, Lehtonen A, Foster D, Julkunen I, and Sareneva T: IL-21 in synergy with IL-15 or IL-18 enhances IFN-$\gamma$ production in human NK and T cells. J Immunol 2003;170:5464–5469.
13. van der Vliet, von Blomberg BM, Hazenberg MD, et al.: Selective decrease in circulating V$\alpha$24+V$\beta$11+ NKT cells during HIV type 1 infection. J Immunol 2003;168: 1490–1495.
14. White L, Krishnan S, Strbo N, et al.: Differential effects of IL-21 and IL-15 on perforin expression, lysosomal degranulation, and proliferation in CD8 T cells of patients with human immunodeficiency virus-1 (HIV). Blood 2007;109: 3873–3880.
15. Zeng R, Spolski R, Finkelstein S, et al.: Synergy of IL-21 and IL-15 in regulating T cell expansion and function. J Exp Med 2005;201:130–148.


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# Article 9

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# Dynamics and Consequences of IL-21 Production in HIV-Infected Individuals: A Longitudinal and Cross-Sectional Study

**Alexandre Iannello,[*,†] Mohamed-Rachid Boulassel,[‡] Suzanne Samarani,[*,†] Olfa Debbeche,[*,†] Cécile Tremblay,[†,§] Emil Toma,[†,§] Jean-Pierre Routy,[‡] and Ali Ahmad[*,†]**

**IL-21 is a relatively newly discovered immune-enhancing cytokine that plays an essential role in controlling chronic viral infections. It is produced mainly by CD4+ T cells, which are also the main targets of HIV-1 and are often depleted in HIV-infected individuals. Therefore, we sought to determine the dynamics of IL-21 production and its potential consequences for the survival of CD4+ T cells and frequencies of HIV-specific CTL. For this purpose, we conducted a series of cross-sectional and longitudinal studies on different groups of HIV-infected patients and show in this study that the cytokine production is compromised early in the course of the infection. The serum cytokine concentrations correlate with CD4+ T cell counts in the infected persons. Among different groups of HIV-infected individuals, only elite controllers maintain normal production of the cytokine. Highly active antiretroviral therapy only partially restores the production of this cytokine. Interestingly, HIV infection of human CD4+ T cells inhibits cytokine production by decreasing the expression of c-Maf in virus-infected cells, not in uninfected bystander cells. We also show that the frequencies of IL-21–producing HIV-specific, but not human CMV-specific, Ag-experienced CD4+ T cells are decreased in HIV-infected viremic patients. Furthermore, we demonstrate in this study that recombinant human IL-21 prevents enhanced spontaneous ex vivo death of CD4+ T cells from HIV-infected patients. Together, our results suggest that serum IL-21 concentrations may serve as a useful biomarker for monitoring HIV disease progression and the cytokine may be considered for immunotherapy in HIV-infected patients.** ***The Journal of Immunology*, 2010, 184: 114–126.**

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Interleukin-21 is a relatively recently discovered multifunctional and pleiotropic cytokine. It is a member of the common γ-chain–using cytokine family that includes IL-2, IL-4, IL-7, IL-9, and IL-15 (1, 2). The cytokine has been shown to exert significant immune-enhancing and immune-regulatory functions. It promotes proliferation and accumulation of Ag-specific CD8+ effector T cells, and increases their survival and cytolytic potential, especially in synergism with other cytokines, such as IL-15 and IL-18 (3, 4). It can also promote differentiation of naive CD4+ T cells into Th17 cells, which play an important role in inducing inflammation and controlling invading pathogens (5, 6). Moreover, the cytokine is needed for homeostatic expansion of Th17 cells (5, 7). IL-21, unlike IL-2, does not support proliferation of anti-CD3–activated regulatory T cells that are involved in suppression of antiviral immunity (8). In fact, the cytokine suppresses expression of FOXP-3, a transcription factor necessary for the development and differentiation of regulatory T cells (9). It is also well known that IL-21 has a significant influence on the regulation of B cell functions: It promotes differentiation of Ag-stimulated B cells into memory and Ab-secreting plasma cells, affects IgE production, and induces Ig switch to IgG1 and IgG3 production (10, 11). This cytokine also inhibits activation and maturation of dendritic cells but increases activation of macrophages and their IL-8 production (12, 13). Several in vivo studies in animal models have shown that IL-21 is essential for controlling chronic viral infections (14–16).

HIV-1 is the etiological agent of AIDS in humans. The infection is usually accompanied by changes in the production of several immunologically important cytokines, such as IL-15, TNF-α, IL-4, IL-12, IL-10, IL-18, and TGF-β1. Today, the production of many of these cytokines is known to be dysregulated in HIV-infected individuals. These cytokine disturbances play an important role in the pathogenesis of AIDS in HIV-infected individuals (17–19). Learning about these disturbances helps in understanding the mechanism of the disease as well as in devising novel, rational, and knowledge-based immunotherapies. Studies have demonstrated that CD4+ T cells are the main producers of IL-21 in the human body (1, 20, 21). It is noteworthy that these cells are also main targets of HIV infection. The virus replicates primarily in CD4+ T cells in HIV-infected individuals. The cells undergo rapid turnover and death even in early asymptomatic stages of the infection (22–24). Depletion of CD4+ T cells in the circulation of HIV-infected individuals is a hallmark of AIDS (25). The depletion of CD4+ T cells is likely to result in a decreased production of IL-21.

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The online version of this article contains supplemental material.

Abbreviations used in this paper: 7-AAD, 7-aminoactinomycin D; CI, chronically infected; CI-H, CI patients receiving HAART; CONT, control; EC, elite controller; HAART, highly active antiretroviral therapy; HCMV, human CMV; LTNP, long-term nonprogressor; MFI, mean fluorescence intensity; NP, normal progressor; PFA, paraformaldehyde; PI, primary infection; rh, recombinant human; siRNA, small interfering RNA; SP, slow progressor.

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Indeed, on the basis of results from a small cross-sectional study, we reported earlier that serum levels of the cytokine were significantly reduced in patients with chronic HIV infection (26). We have now performed much larger cross-sectional and longitudinal studies to better understand the dynamics of cytokine production in different stages of the infection. In this paper, we show that concentrations of this cytokine are decreased in the circulation of HIV-infected individuals soon after infection and in general follow the kinetics of the $CD4^+$ T cell counts of infected persons in all groups. Interestingly, we found that the elite controllers (EC; see below) maintain normal levels of the cytokine in their circulation. Our results also show that highly active antiretroviral therapy (HAART) causes a partial restoration of IL-21 levels in these patients. More importantly, we have discovered that in vitro infection of human PBMCs and purified $CD4^+$ T cells with the virus reduces their production of IL-21 at both mRNA and protein levels. The infection reduces expression of the transcription factor c-Maf, which is known to play a key role in transcriptional activation of the *IL-21* gene in $CD4^+$ T cells (27). We found decreased frequencies of IL-21–producing HIV-specific Ag-experienced $CD4^+$ T cells in HIV-infected viremic patients, compared with frequencies of the cells specific for the human CMV (HCMV). We also provide experimental evidence to indicate that IL-21 protects primary human $CD4^+$ T cells in vitro from undergoing spontaneous apoptosis. In vitro, the cytokine restores survival of $CD4^+$ T cells from HIV-infected patients comparable to survival of cells from HIV-seronegative healthy subjects. Finally, we demonstrate that decreased serum concentrations of IL-21 have consequences for the frequency and functionality of HIV-specific CTL in HIV-infected individuals. These results suggest that a reduced production of IL-21 in HIV-infected persons may be at least one reason for the decreased survival of their $CD4^+$ T cells and may adversely affect their antiviral CTL responses as well.

## Materials and Methods

### *Cell cultures and HIV infection*

All cells used in this study were cultured at 37˚C in humidified atmosphere containing 5% $CO_2$. PBMCs were obtained from the peripheral blood of donors by centrifugation over a Ficoll-Hypaque (Pharmacia, Montreal, Quebec, Canada) cushion, as described earlier (28). After washing with PBS, the cells were cultured in RPMI 1640 culture medium (Life Technologies, Burlington, Ontario, Canada) supplemented with 10% heat-inactivated FBS. The cells were activated with PHA (10 μg/ml) and IL-2 (100 U/ml) for 3 d. For some experiments, human $CD4^+$ T cells were isolated by negative selection using a kit (Stem Cell Technology, Vancouver, British Columbia, Canada). The purified $CD4^+$ T cells, which were >96% positive for CD4 expression, were infected in vitro with a T-tropic HIV-1 strain (NL4.3). The cells were infected for 2 h at 37˚C with a multiplicity of infection of 1 and were extensively washed with the culture medium to remove residual virus. The infected cells were incubated at 37˚C for different lengths of time.

### *Abs, reagents, and recombinant cytokines*

The Abs used in this study were purchased and included the following: FITC-conjugated mouse anti-CD3, APC-conjugated mouse anti-CD4, PE-conjugated mouse anti-CD3, anti-IL-2, anti-IL-4, anti-IL-21 and anti-IFN-γ from eBioscience (San Diego, CA), FITC-conjugated mouse anti-HIV–1 p24 from Thermo Scientific (Rockford, IL), FITC-conjugated mouse anti-CD4, PE-conjugated mouse anti-human IL-21Rα chain and 7-aminoactinomycin D (7-AAD) from BD Biosciences (Mississauga, Ontario, Canada), rabbit anti-human c-Maf from Santa Cruz Biotechnology (Santa Cruz, CA), FITC-conjugated goat anti-rabbit IgG from Sigma-Aldrich (St. Louis, MO), and mouse anti-human GAPDH from Ambion (Austin, TX). The recombinant human (rh) cytokines used for this study were also purchased: rhIL-2 from Hoffmann-La Roche (Mississauga, Ontario, Canada) and rhIL-21 from BioSource International (Camarillo, CA). PHA-L, brefeldin A, and ionomycin were purchased from Sigma-Aldrich, and 3TC was obtained from the AIDS and Cancer Virus Program (National Cancer Institute, Frederick, MD).

### *Patients and sera collection*

A total of 127 serum samples from a cross section of HIV-infected AIDS patients were used in this study. These patients represented different durations of the infection and included 32 patients with primary infection (PI; defined as being within 6 mo of date of infection and receiving no HAART), 41 chronically infected (CI) patients (having infection for >6 mo and naive for HAART), 34 CI patients receiving HAART (CI-H), and 20 long-term nonprogressors (LTNP; infected for >7 y, not receiving HAART, and without having any AIDS-defining condition; Table I). LTNP could be further classified into three subgroups, depending on their viral loads and $CD4^+$ T cell counts: slow progressors (SP; with viral load between 51 and 3000 copies/ml), normal progressors (NP; with viral load between 3,000 and 50,000 copies/ml), and EC (with CD4 > 500 cells/$mm^3$ and viral load < 50 copies/ml). Sera from 38 adult HIV-seronegative healthy subjects were used as controls for this study. $CD4^+$ T cells were enumerated by flow cytometry, according to the quality assessment program for T cell subset enumeration (Canadian National Laboratory for HIV-1 Analytical Cytology, Ottawa, Ontario, Canada). Plasma viral load was measured using the Roche Amplicor Assay (Roche Diagnostics, Laval, Quebec, Canada). The date of infection was determined following the guidelines proposed by the Acute HIV Infection and Early Disease Research Program sponsored by the Division of AIDS of the National Institute of Allergy and Infectious Disease. The study was approved by the Institutional Ethics Committee, and peripheral blood samples were obtained from the study participants after their written and informed consent.

### *Measuring IL-21 concentrations*

The concentrations of IL-21 were determined in serum samples and culture supernatants, using a commercial ELISA kit (eBioscience). The lower detection limit of the kit was 31 pg/ml.

### *Flow cytometry*

For this purpose, $10^6$ cells were incubated with fluorochrome-conjugated Abs for 45 min on ice and washed three times with PBS containing 0.05% BSA and 0.002% sodium azide. Intracellular stainings were performed with the Cytofix/Cytoperm Kit (BD Biosciences). The washed cells were resuspended in 2% paraformaldehyde (PFA) and analyzed by flow cytometry using a FACSCalibur (BD Biosciences). CellQuest Pro software (BD Biosciences) was used to collect and analyze the flow cytometry data.

### *Western blotting*

The expression of different proteins was analyzed by Western blotting, as described in our earlier publications (29). Briefly, $5 \times 10^6$ cells were incubated in the culture medium with and without treatment, as detailed in individual experiments. At different time points postincubation, the cells were washed with PBS and lyzed in a lysis buffer containing Tris HCl (pH 6.8; 50 mM), SDS (2%), leupeptin (1 mg/ml), PMFS (1 mM), and pepstatin (1 mg/ml). The lysates were clarified by centrifugation at 14,000 × g for 15 min. Protein concentrations in the lysates were determined using a commercial kit (Pierce, Nepean, Ontario, Canada). Forty micrograms of the lysate proteins were mixed with 2× SDS-PAGE sample loading buffer containing 1 mM dithiothreitol, boiled, run on 12% polyacrylamide gels, and electroblotted onto polyvinylidene difluoride membranes (Immobilon-P; Millipore, Ontario, Canada). After the membranes were blocked in 1% casein for 2 h at room temperature, they were incubated on a shaker with human protein-specific Abs at 4˚C overnight. The protein bands were revealed by autoradiography using biotinylated secondary Abs and a commercial chemiluminescent kit (Vectastain ABC-AmP; Vector Labs, Burlington, Ontario, Canada). Individual bands on the x-ray films were quantified by densitometry.

### *Real-time quantitative RT-PCR*

The method was used to measure IL-21 mRNA in cells. For this purpose, total RNA was extracted from the cells with a kit (RNeasy Micro Kit, Qiagen, Ontario, Canada), and cDNA was synthesized using the QuantiTect Reverse Transcription Kit (Qiagen). The QuantiTect SYBR Green PCR Kit (Qiagen) was used to analyze cDNA by real-time quantitative PCR with a set of primers for the human *IL-21* gene. The primers were designed across introns of the gene and were as follows: forward 5′-ACTGGTCCACAAATCAAGCTCCCA-3′ and reverse 5′-TTCTGGAGCTGGCAGAAATTCAGG-3′. Samples for RT-PCR were run on the SmartCycler Mx3000P (Stratagene, La Jolla, CA), and expression of the *IL-21* gene was normalized to that of a human housekeeping gene, *GAPDH*. The primers used to amplify *GAPDH* were as follows: forward 5′-ATCGTGGAAGGACTCATGACCACA-3′ and reverse 5′-TAGAGGCAGGGATGATGTTCTGGA-3′. Error bars were calculated based on triplicate measurements for each gene.

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*siRNA and transfection*

In some experiments, small interfering RNA (siRNA) was used to inhibit expression of c-Maf in purified human $CD4^+$ T cells. Five million $CD4^+$ T cells isolated from PBMCs were transfected with 30 pmol of the c-Maf–specific or control siRNA duplexes (Santa Cruz Biotechnology), using a commercial transfection kit and the Nucleofector device (T Cell Nucleofector Kit; Amaxa, Gaithersburg, MD) following the supplier's instructions. The protocol U-014, which ensures high viability, was used.

*Quantification of IL-21–producing Ag experienced HIV- and HCMV-specific $CD4^+$ T cells*

Fifty million frozen PBMCs from each of HAART-naïve, HIV-infected viremic patients (five) and HIV-seronegative healthy donors (five) were rapidly thawed and incubated with PBS, HCMV lysate, or HIV-1 lysate. The viral lysates were obtained from Advanced Biotechnologies (Columbia, MD) and were used at 10 μg/ml. Anti-CD28 and anti-CD49d Abs (each at 1 μg/ml; from eBioscience) were added to the cultures. The cultures were incubated at 37°C in humidified 5% $CO_2$ atmosphere for 10 h. Brefeldin A (1 μg/ml) was added to the cultures in the last 4 h of the incubation period. Postincubation, the cells were washed with PBS, and $CD4^+$ T cells were isolated by negative selection using a commercial kit (Stem Cell Technology). The isolated $CD4^+$ T cells (96% pure or more) were stained with APC-conjugated anti-CD27, FITC-conjugated anti-CCR7 (both from eBioscience), and PerCP-conjugated anti-CD45RA (BioLegend, San Diego, CA). The stained cells in each treatment were divided into two aliquots and were stained intracellularly using Cytofix/Cytoperm Kit (BD Biosciences) with PE-conjugated IL-21 (eBioscience) or with PE-conjugated IFN-γ (eBioscience). The cells were washed, resuspended in 2% PFA, and analyzed by flow cytometry using FACSCalibur (BD Biosciences). Live cells were gated for three different Ag-experienced phenotypes ($CD27^+CD45RA^-CCR7^+$, $CD27^+CD45RA^-CCR7^-$ or $CD27^-CD45RA^-CCR7^-$) (30) and analyzed for the intracellular expression of IL-21 or IFN-γ. For each phenotype, $10^5$ events were acquired.

*Measuring frequencies and functionality of HIV-specific CTL*

For this purpose, we selected six HIV-infected individuals who had high (155, 210, and 189 pg/ml) or low (31, 18, and 29 pg/ml) serum concentrations of IL-21. These patients were viremic and HAART naive. Ten million PBMCs from each of the HIV-infected individuals and three HIV-seronegative healthy donors were rapidly thawed, washed with sterile PBS (pH 7.3), and incubated in the culture medium containing vehicle (DMSO) or 0.20 μg/ml of each of the 48 20-mer overlapping peptides corresponding to the entire amino acid sequence of the HIV-1 (HxB-2) Gag protein (obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Disease, National Institutes of Health; Catalogue # 3992). Anti-CD28 and anti-CD49d Abs (each at 1 μg/ml; from eBioscience) were added to the cultures. The cultures were incubated at 37°C in humidified 5% $CO_2$ atmosphere for 10 h. Brefeldin A (1 μg/ml) was added to the cultures in the last 4 h of the incubation period. Postincubation, the cells were washed with PBS and stained with FITC-conjugated anti-CD8 and PE-Cy7-conjugated anti-CD3 Abs (both from eBioscience). The cells were also stained intracellularly using the Cytofix/Cytoperm Kit (BD Biosciences) with APC-conjugated anti-IL-2 and PE-conjugated IFN-γ Abs (both from eBioscience). The cells were then washed with PBS, resuspended in PBS containing 2% PFA, and analyzed by flow cytometry using FACSCalibur (BD Biosciences). Live cells were gated for $CD8^+CD3^+$ T cells and analyzed for frequencies of the cells expressing IL-2, IFN-γ and both IL-2 and IFN-γ from $10^5$ events.

*Statistical analysis*

Group means were compared using ANOVA, and Pearson's correlation between two parameters was determined with the software PRISM (GrapPad, San Diego, CA). Differences and correlations were deemed significant at $p \leq 0.05$.

## Results

*Levels of circulating IL-21 are decreased in HIV-infected patients*

First, we conducted a cross-sectional study and measured serum levels of IL-21 in 127 sera from HIV-infected patients and in 38 sera from age-matched HIV-seronegative healthy subjects. The serum samples obtained from HIV/AIDS patients represented different disease conditions, as described in Table I in *Materials and Methods*. The average concentrations of the cytokine for the control and different groups of the patients are shown in Fig. 1*A*. Sera from PI patients showed significantly decreased ($p < 0.001$) levels of IL-21, compared with sera from healthy subjects (91 ± 54 versus 496 ± 189 pg/ml, respectively). The average concentration in this infected group sera was approximately one-fifth that of the control sera. Similarly, significantly decreased ($p < 0.001$) levels of IL-21 were observed for chronic HIV infection patients naive for HAART, compared with healthy subjects (56 ± 29 versus 496 ± 189 pg/ml, respectively). The average IL-21 level in this infected group sera was approximately one-ninth that of the control sera. Decreased levels of the cytokine were also observed in CI-H patients, compared with healthy subjects (303 ± 143 versus 496 ± 189 pg/ml, respectively). The difference between mean levels of IL-21 for these two groups of sera was statistically significant ($p < 0.001$). Interestingly, the average IL-21 level of the group receiving HAART was ~3–4-fold higher than that of CI patients who were naive for HAART. Sera from the HAART-receiving patients also showed ~3–4-fold higher average IL-21 content, compared with the average value for sera from PI patients. These results suggest positive restorative effects of the antiviral treatment on the serum levels of IL-21. Nevertheless, levels of IL-21 in patients receiving HAART still remained significantly lower ($p < 0.001$) compared with those of healthy donors, suggesting that HAART causes a partial restoration of IL-21 production in infected persons. Of note, the average serum IL-21 level for the LTNP group was significantly higher ($p < 0.001$) than that for either CI or PI patients. The average levels, however, were lower than those of the control group and patients who had received HAART (Fig. 1*A*). The median values of cytokine concentration, as well the quartile ranges for the study participants, are shown in Supplemental Fig. 1.

On the basis of viral loads and $CD4^+$ T cell counts, we classified LTNP into three groups–NP, SP, and EC–as described in *Materials and Methods*. A comparison of cytokine levels in the sera of these groups is shown in Fig. 1*B*. A progressive increase in the average serum levels of the cytokine from NP to EC is quite evident. These levels are lower in NP and SP than in healthy control subjects ($p < 0.001$). Interestingly, IL-21 concentrations in the EC were not significantly different from those in healthy subjects

Table I. *HIV-infected patients*

| Category | No. of Patients | Main Clinical Features | |
|---|---|---|---|
| | | A[a] | B[a] |
| PI | 32 | Median: 381<br>Range: 3–854 | Median: 29,799<br>Range: 156–511,143 |
| CI | 41 | Median: 312<br>Range: 92–1261 | Median: 28,348<br>Range: 267–500,000 |
| CI-H | 34 | Median: 303<br>Range: 86–653 | Median: 2400<br>Range: 500–457,000 |
| LTNP | | | |
| NP | 6 | Median: 545<br>Range: 400–990 | Median: 32,500<br>Range: 4228–50,125 |
| SP | 8 | Median: 613<br>Range: 400–1150 | Median: 970<br>Range: 90–9314 |
| EC | 6 | Median: 810<br>Range: 430–850 | <50 |

The infection was defined PI if it had been within 6 mo of the time of seroconversion and CI if it had been >6 mo.

[a]A and B indicate $CD4^+$ T cell counts (cells/μl) and viral loads (HIV-1 RNA copies/ml), respectively.

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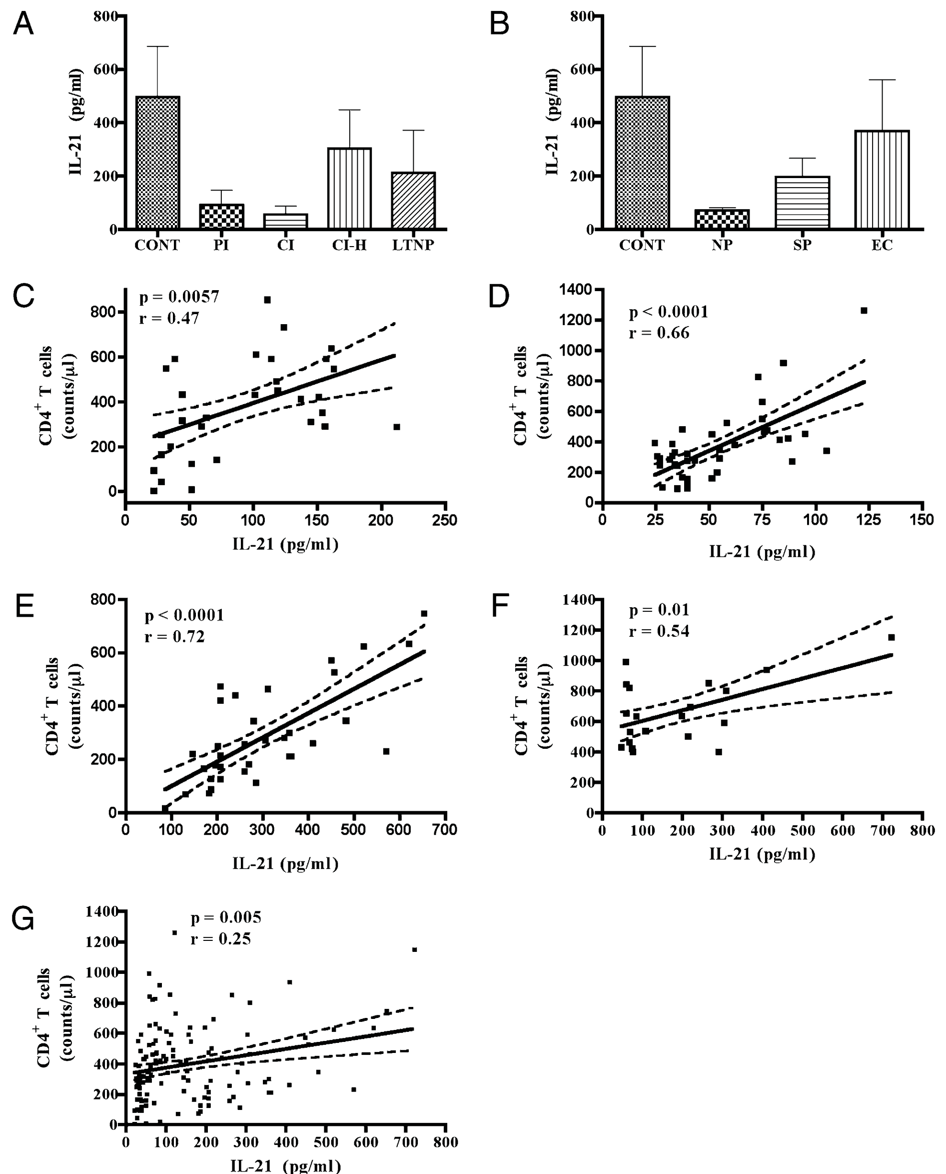


**FIGURE 1.** Serum IL-21 concentrations in HIV-infected patients. IL-21 concentrations were determined in serum samples using a commercial ELISA kit. *A*, Average concentrations ±SD of IL-21 in the sera of 38 control (CONT) subjects, 32 PI patients, 41 CI patients, 34 CI-H, and 20 LTNP. Average concentrations differed significantly between the different infected groups of patients and CONT subjects ($p < 0.0001$). *B*, Average concentrations ±SD of IL-21 in the sera of 38 CONT subjects and the three groups of LTNP representing 6 SP, 8 NP, and 6 EC. Average concentrations of the CONT group differed significantly from those of NP and SP donors ($p < 0.0001$). Pearson's correlation between concentrations of IL-21 and $CD4^+$ T cell counts is given for PI patients (*C*), CI patients naive for HAART (*D*), CI-H (*E*), LTNP (*F*), and all HIV-infected patients (*G*). The two parameters showed a statistically significant correlation for all groups of patients: ($p = 0.0057$; $r = 0.47$ for PI), ($p < 0.0001$; $r = 0.66$ for CI), ($p < 0.0001$; $r = 0.72$ for CI-H), ($p = 0.01$; $r = 0.54$ for LTNP), and ($p = 0.005$; $r = 0.25$ for all patients). The dashed lines indicate 90% confidence limit.

($p > 0.05$) and were ~5-fold higher than those of NP (370 ± 191 versus 71 ± 10 respectively; $p < 0.05$). The average IL-21 concentration in EC was also higher than that in SP (370 ± 191 versus 197 ± 70 pg/ml, respectively; $p < 0.05$). These data suggest that serum IL-21 concentrations remain elevated in HIV-infected patients who are efficiently controlling the infection. This finding may indicate a role for this cytokine in antiviral immunity and in delaying AIDS progression.

$CD4^+$ T cells are recognized as the main producers of IL-21 in the human body. The cell numbers are known to decrease in HIV-infected individuals. This prompted us to determine whether a correlation existed between the serum concentrations of this cytokine and $CD4^+$ T cell counts in these patients. As shown in Fig. 1*C–F*, we found significant positive correlations between the two parameters for all groups of patients: ($p = 0.0057$; $r = 0.47$ for PI patients; Fig. 1*C*), ($p < 0.0001$; $r = 0.66$ for CI patients; Fig. 1*D*), ($p < 0.0001$; $r = 0.72$ for CI-H; Fig. 1*E*), and ($p = 0.01$; $r = 0.54$ for LTNP; Fig. 1*F*). Correlations between cytokine levels and $CD4^+$ T cell numbers were also significant ($p = 0.005$; $r = 0.25$) when we considered the two parameters for all HIV-infected patients irrespective of infection stage and use of antiretroviral therapy (Fig. 1*G*). The correlations, however, were nonsignificant ($p > 0.05$) between IL-21 levels and viral loads and between serum cytokine levels and total $CD8^+$ T cell counts (also see below) for all groups of patients (data not shown).

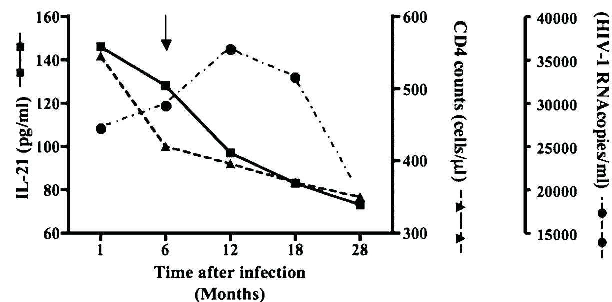


**FIGURE 2.** Kinetics of serum IL-21, $CD4^+$ T cell counts, and viral loads in treatment-naive HIV-infected patients after start of infection. Serum IL-21 concentrations from nine patients naive for HAART were determined at indicated time points from their day of infection (time zero) until 28 mo. Shown are median values from nine patients for serum IL-21, $CD4^+$ T cell counts and plasma viral loads. The arrow in the graph indicates end of duration of PI (6 mo postinfection).

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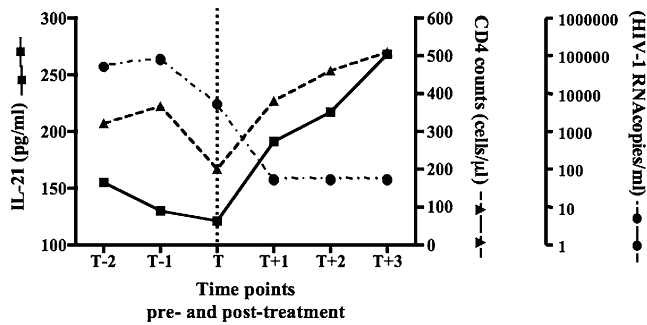


**FIGURE 3.** Kinetics of serum IL-21, $CD4^+$ T cell counts, and viral loads in CI patients before and after HAART. IL-21 concentrations, $CD4^+$ T cell counts, and plasma viral loads were determined longitudinally at the indicated time points for 28 mo after the day of infection. The vertical dotted line indicates end of duration for PI (6 mo postinfection). The letter T on the *x*-axis indicates start of HAART. Shown are median values for serum IL-21 levels, $CD4^+$ T cell counts, and plasma viral loads. T+1, T+2, and T +3 indicate first, second, and third time points for serum collection after initiation of HAART. T-1 and T-2 indicate time points prior to initiation of HAART.

*IL-21 concentrations start decreasing early in the course of primary HIV infection and correlate positively with $CD4^+$ T cell counts*

We investigated the kinetics of IL-21 production in treatment-naive HIV-infected patients by analyzing their sera taken longitudinally at different time points (until 28 mo) after the date of their infection (time zero). These patients did not belong to the cohort who were participants in the cross-sectional study mentioned above. Fig. 2 shows median values from nine patients for serum IL-21, $CD4^+$ T cell counts, and plasma viral loads. Mean values of these parameters as well as values for each of the patients are given in Supplemental Figs. 2 and 3, respectively. These data show that IL-21 concentrations start decreasing early in the course of the PI. In almost all cases, cytokine concentrations decreased progressively over time. The decrease in IL-21 levels is accompanied by a concomitant decline in the numbers of $CD4^+$ T cells. Serum cytokine levels and viral loads, however, showed no consistent trend with respect to each other in untreated HIV-infected patients. Collectively, these data suggest that IL-21 levels decrease progressively with time, starting early in the course of the infection, and are accompanied by loss of $CD4^+$ T cells.

*HAART stops decreases in serum IL-21 concentrations and increases them progressively after beginning treatment*

To investigate the impact of HAART on IL-21 concentrations in the serum, we followed up and measured cytokine levels in the sera of eight HIV-infected patients before and after HAART. The changes observed in serum IL-21 concentrations, plasma viral loads, and $CD4^+$ T cell counts are summarized in Fig. 3. Mean values and values for each HIV-infected individual are shown in Supplemental Figs. 4 and 5, respectively. These data suggest that levels of the cytokine increased progressively with time after beginning

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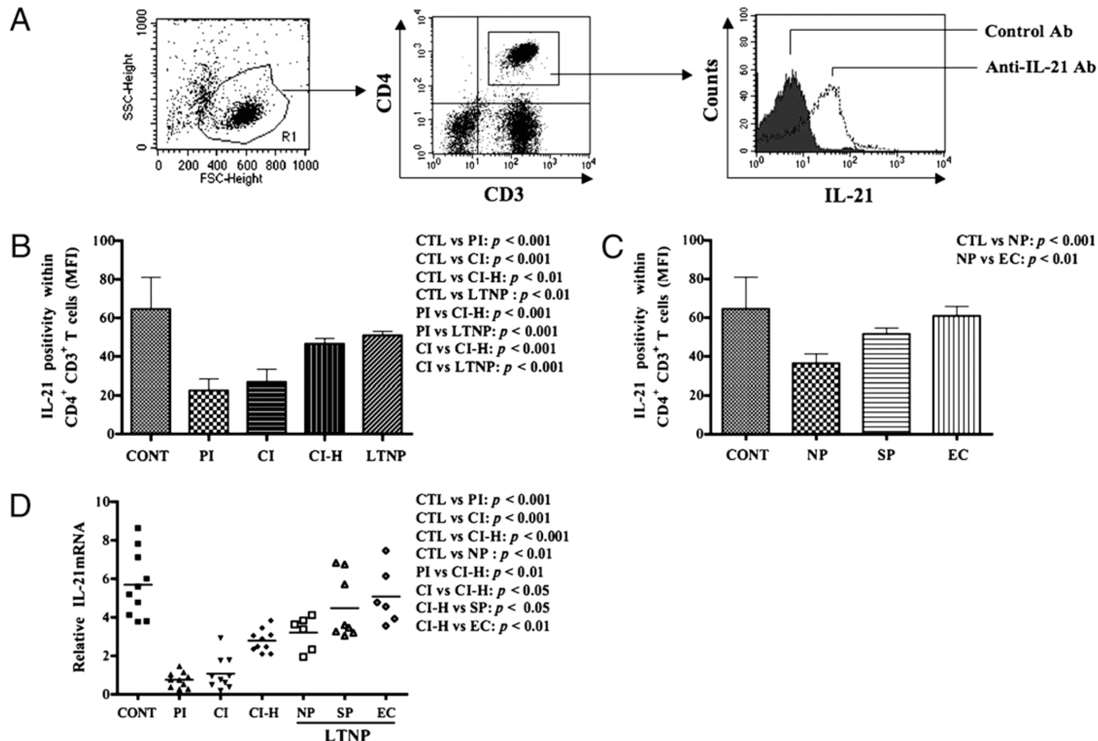


**FIGURE 4.** IL-21 production per $CD4^+$ T cell decreases in HIV-infected patients. *A*, PBMCs from 10 patients in each group (PI, CI, and CI-H), 20 LTNP (6 SP, 8 NP, and 6 EC), and 10 healthy CONT subjects were incubated ex vivo in the culture medium containing ionomycin (1 µg/ml) for 24 h. After 24 h, the cells were treated with brefeldin A (1 µg/ml) for 4 h, washed, and stained with FITC-conjugated anti-CD3 and APC-conjugated anti-CD4 Abs. The cells were washed, permeabilized, and stained with PE-conjugated anti-IL-21 Abs. IL-21 expression was measured by flow cytometry from 10,000 events gated on live $CD3^+CD4^+$ cells. Shown in *A* are gating for $CD3^+CD4^+$ T cells and intracellular staining for PE-conjugated isotype-matched Abs and for PE-conjugated anti–IL-21 Abs. *B*, Averages ±SD of mean fluorescence intensities (MFI) of IL-21–positive cells in different groups of donors. *C*, The MFI values for healthy CONT subjects, NP, SP, and EC patients. The average MFI of the CONT group differed significantly only from that of the NP donors ($p < 0.001$). *D*, IL-21 mRNA from 1 million PBMCs was measured by real-time RT-PCR in 10 subjects from each group of donors. The mRNA expression was normalized to GAPDH mRNA levels. IL-21 mRNA is shown in arbitrary units for each group of study participants. Important significantly different pairs of means are shown on the right side of the panels.

antiviral treatment. This rise in IL-21 concentration is accompanied by an increase in the number of $CD4^+$ T cells in most patients posttherapy. Interestingly, at the time points at which IL-21 concentrations start augmenting posttreatment, we also observe the beginning of a decline in plasma viral loads in these patients.

### *$CD4^+$ T cells from HIV-infected patients have a decreased ability to produce IL-21*

A significant positive correlation between $CD4^+$ T cell counts and serum IL-21 concentrations, observed above, suggests that a depletion of $CD4^+$ T cells plays a role in decreased serum concentrations of the cytokine. However, decreased serum concentrations may also result from reduced production of the cytokine by $CD4^+$ T cells. Therefore, we compared the cytokine-producing capacities of cells from HIV-infected subjects and from healthy subjects. For this purpose, we isolated PBMCs from HIV-infected and healthy control subjects, then cultured them in vitro in the presence of ionomycin (1 μg/ml). Next, we stained the cells for CD3, CD4, and intracellular IL-21 and examined them by flow cytometry, as shown in Fig. 4*A*. As seen in Fig. 4*B,* the expression of IL-21 was very low in PI and CI patients, compared with healthy subjects ($p < 0.001$). The average level of the cytokine in CI-H patients was elevated. Nevertheless, the levels of IL-21 still remained significantly lower ($p < 0.01$) in patients receiving HAART than in healthy donors. The average IL-21 level for LTNP was significantly higher ($p < 0.01$) than that for either the CI or the PI patients. A progressive increase in the average intracellular levels of the cytokine in $CD4^+$ T cells from NP to EC was also observed (Fig. 4*C*). The SP and EC patients also had elevated levels of IL-21 in $CD4^+$ T cells, compared with those seen in other groups (Fig. 4*C*). No positive signal for intracellular IL-21 was observed in $CD3^+CD4^-$ and $CD3^-$ cells in the PBMCs from HIV-infected as well as from HIV-seronegative healthy individuals (data not shown).

We also measured IL-21 mRNA expression by quantitative real-time RT-PCR in PBMCs obtained from different groups of patients, and normalized it with respect to the mRNA of a housekeeping gene, *GAPDH*. As shown in Fig. 4*D,* the expression of IL-21 mRNA is very low in PI and CI patients compared with healthy subjects ($p < 0.001$). The average level of the cytokine mRNA in CI-H patients was elevated and was not statistically different ($p > 0.05$) from that found in NP patients. Of note, SP and EC patients, compared with other groups of patients, had elevated levels of the cytokine mRNA. Again, EC expressed the same amount of IL-21 mRNA as that seen in healthy donors (Fig. 4*D*). In general, the IL-21 mRNA-producing abilities of the PBMCs are in accord with intracellular expression of the cytokine in $CD3^+CD4^+$ T cells in all groups of donors. Taken together, these data suggest that low serum levels of IL-21 are also due to an attenuated ability of the PBMCs, and more specifically of $CD3^+CD4^+$ T cells, to produce IL-21 in HIV-infected patients. The results also indicate that these cells in the EC group maintain their ability to produce IL-21 at normal levels.

### *HIV infection decreases the ability of human $CD4^+$ T cells to produce IL-21*

The decreased ability of $CD4^+$ T cells to produce IL-21 in HIV-infected individuals prompted us to investigate the effect of HIV infection on these cells. For this purpose, we performed in vitro infection of human $CD4^+$ T cells purified from PBMCs after their activation with PHA and IL-2 for 72 h. The PBMCs were obtained from HIV-seronegative healthy donors. The cells were infected with a T-tropic HIV-1 strain (NL4.3) with a multiplicity of infection of 1. The infected cells were cultured for different lengths of time in the presence of ionomycin (1 μg/ml). At each time point, the numbers of viable cells were counted by Trypan blue exclusion, and the culture supernatants were collected and centrifuged to remove debris. The IL-21 concentrations of the

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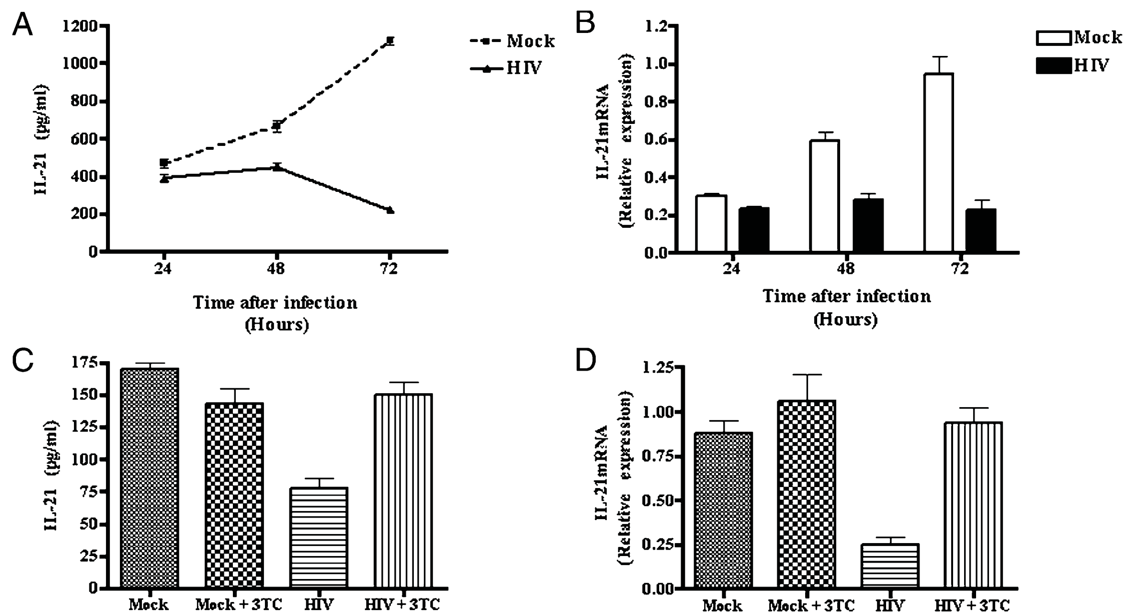


**FIGURE 5.** Effect of HIV infection on IL-21 production in $CD4^+$ T cells. *A*, Human $CD4^+$ T cells were purified from PHA and IL-2–activated PBMCs, infected in vitro with a T-tropic HIV strain (NL4.3), washed, and cultured for different lengths of time in the presence of ionomycin (1 μg/ml) with and without 3TC (200 nM). The IL-21 secretion was measured in the culture supernatants with an ELISA kit. At each time point, the numbers of live cells were counted in the microcultures. The cytokine concentration was normalized with the number of live $CD4^+$ T cells at each point. Concentrations of the cytokine in the culture supernatants are shown at the indicated time points. *B*, IL-21 mRNA levels in HIV-infected and mock-infected $CD4^+$ T cells normalized to the GAPDH mRNA levels and the number of live $CD4^+$ T cells in arbitrary units. *C*, Effect of in vitro HIV infection and 3TC (200 nM) on IL-21 production (72 h postinfection) from human $CD4^+$ T cells. The cytokine concentration was normalized with the number of live $CD4^+$ T cells. *D*, Effect of the infection and 3TC on IL-21 mRNA expression in human $CD4^+$ T cells normalized to the GAPDH mRNA levels and the number of live $CD4^+$ T cells in arbitrary units. Essentially, similar results were obtained with three different healthy donors.

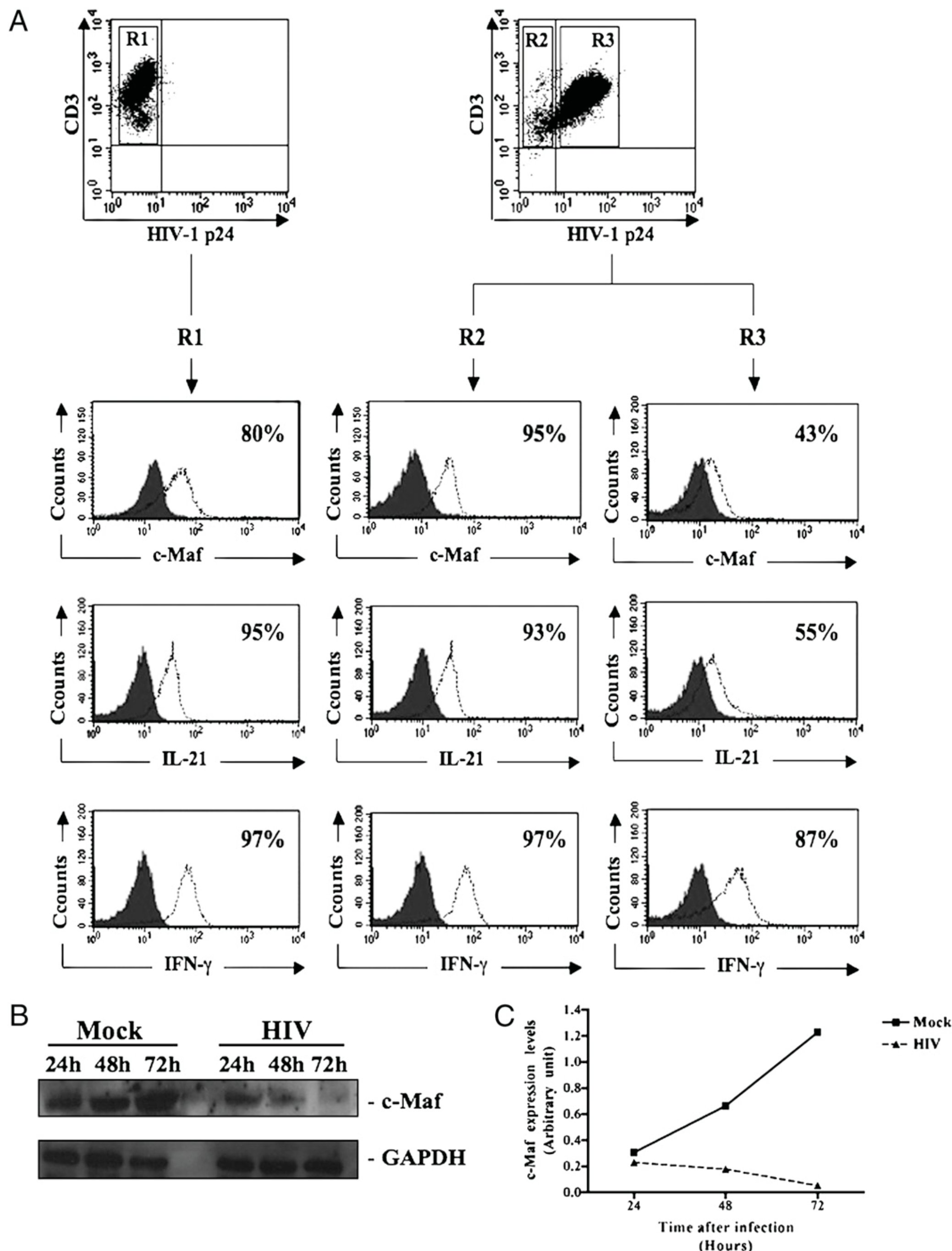


**FIGURE 6.** HIV infection reduces expression of the transcription factor c-Maf. *A*, Human $CD4^+$ T cells were isolated from human PBMCs after 72 h activation with PHA (10 μg/ml) and IL-2 (100 U/ml). The cells were infected in vitro with a T-tropic HIV strain (NL4.3) and cultured at 37°C in humidified 5% $CO_2$ atmosphere in the presence of ionomycin (1 μg/ml) for 48 h. Brefeldin A (1 μg/ml) was added to the cell cultures for the last 2 h. Postincubation, the cells were harvested, washed, and stained extracellularly for CD3 and intracellularly for HIV-1 p24 and IL-21, c-Maf, or IFN-γ. The upper two panels show the gating strategy. R1 indicates $CD3^+p24^-$ cells selected from mock-infected cell cultures. R2 and R3 indicate the gates for $CD3^+p24^-$ and $CD3^+p24^+$ cells from HIV-infected cell cultures, respectively. The gated cells were analyzed for expression of intracellular IL-21, c-Maf, and IFN-γ. For each analysis, 10,000 gated cells were analyzed. The shaded area in each histogram indicates staining with an isotype-matched control Ab. The percentage of cells positive for each marker is indicted in the respective panel. Note a downregulation of IL-21 and c-Maf but not of IFN-γ in $CD3^+p24^+$ cells. *B*, Western blots for expression of c-Maf and GAPDH in lysates from HIV-infected and mock-infected $CD4^+$ T cells at the indicated time points. *C*, Ratios between c-Maf and GAPDH expression after densitometric measurements of the Western blot bands shown in *B*. Essentially similar results were obtained with three different healthy donors.

culture supernatants were measured by ELISA, and IL-21 mRNA was measured by real-time RT-PCR. As shown in Fig. 5*A,* we observed a progressive decrease of IL-21 secretion from HIV-infected PBMCs, especially 48 h ($p < 0.01$) and 72 h postinfection ($p < 0.001$). We also measured IL-21 mRNA expression in vitro postinfection at each time point and observed a progressive decline in IL-21 mRNA production in the infected cells ($p < 0.001$) (Fig. 5*B*). To determine whether HIV replication was necessary for decreased IL-21 production, we infected PBMCs with and without 3TC; a nucleoside reverse transcriptase inhibitor known to block viral replication (31). The treatment with 3TC restored IL-21 production in the cell culture supernatants ($p < 0.01$) as well as

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IL-21 mRNA production in PBMCs ($p < 0.001$) (Fig. 5*C*, 5*D*). A reduced production of the cytokine was also observed when activated PBMCs from HIV-seronegative healthy subjects were infected in vitro with HIV-1 (shown in Supplemental Fig. 6). Taken together, these data suggest that HIV replication has a direct suppressive effect on IL-21 production in CD4$^+$ T cells at mRNA and protein levels.

*HIV infection decreases the expression of the transcription factor c-Maf*

We next wanted to investigate the mechanism involved in the HIV-induced downregulation of IL-21 in CD4$^+$ T cells. Recent studies have shown that expression of c-Maf, the cellular homolog of the avian viral oncogene *v-Maf* and the first identified Th2-type cytokine-specific transcription factor, is needed for activation of the *IL-21* gene in CD4$^+$ T cells (27, 32). The lack of expression of this factor has been shown to cause a drastic downregulation of IL-21 production in CD4$^+$ T cells (27). Therefore, we sought to determine whether HIV infection affected c-Maf expression. To this end, we performed in vitro infection of CD4$^+$ T cells purified from PHA and IL-2–activated PBMCs obtained from healthy donors with HIV-1, as described in *Materials and Methods*. After 24 h, the cells were analyzed for their intracellular expression of cMaf, IFN-γ, and IL-21. As shown in Fig. 6*A,* infection with HIV-1 decreased the production of c-Maf as well as of intracellular IL-21, compared with mock-infected cells, but not of intracellular IFN-γ. We also verified the decreased expression of c-Maf in the infected cells by Western blots at different time points postinfection (Fig. 6*B*). In these experiments, we measured the expression of c-Maf relative to the expression of the housekeeping gene *GAPDH* by densitometry of their respective bands. The ratios between the expression of *IL-21* and *GAPDH* at each time point are depicted in Fig. 6*C*. These data clearly show that the expression of c-Maf increased with time in mock-infected cells, which correlates with a progressive increase in IL-21 levels secreted in the cell supernatants (Fig. 5*A*). Note that these cells have been cultured in the presence of ionomycin. However, the infection caused a progressive decrease in the expression of this factor in purified CD4$^+$ T cells. Fig. 6*A* also shows that HIV infection of CD4$^+$ T cells does not affect their IFN-γ production. Furthermore, it reveals that in HIV-infected CD4$^+$ T cell cultures, noninfected cells (CD3$^-$ p24$^-$ cells) retain their ability to produce IL-21 and maintain normal expression of c-Maf. Taken together, these data suggest that HIV-induced decreased expression of IL-21 is mediated, at least in part, by decreased expression of c-Maf, and it happens only in virus-infected cells, not in noninfected bystander cells.

*Inhibition of c-Maf expression by siRNA downregulates intracellular expression of IL-21*

We further sought to determine whether inhibition of c-Maf expression results in decreased expression of IL-21 in human CD4$^+$ T cells. For this purpose, we isolated human CD4$^+$ T cells from PBMCs obtained from HIV-seronegative healthy subjects. We transfected them with a c-Maf–specific or control siRNA (both from Santa Cruz Biotechnology), as described in *Materials and Methods*. Transfection of the siRNA vector resulted in ~80–90% decrease in the expression of c-Maf in the transfected cells (Fig. 7*A* and data not shown). We cultured the transfected cells in vitro in the presence of ionomycin (1 μg/ml) for 24 h and then harvested the cells. The cells were washed and divided into aliquots. The aliquots were stained intracellularly for IL-21, IL-2, or IL-4. As shown in Fig. 7*B,* the c-Maf siRNA–transfected cells downregulated their expression of IL-21 and IL-4, but not of IL-2. These and previous results suggest that c-Maf–specific siRNA and HIV-1 infection both induced downregulation of c-Maf, which specifically downregulated the expression of c-Maf–regulated genes (IL-21 and IL-4) but not of c-Maf–unaffected genes (IL-2 and IFN-γ).

*Loss of IL-21–producing HIV-specific CD4$^+$ T cells in HIV-infected individuals*

We compared percentages of IL-21– and IFN-γ–producing Ag-experienced HIV-1 and HCMV-specific CD4$^+$ T cells after in vitro stimulation of PBMCs with viral lysates, as detailed in *Materials and Methods*. The results from five HIV-infected HAART-naive viremic individuals (different from those participating elsewhere in this study) and five HIV-seronegative healthy controls are shown in Table II. The mean percentages of all subsets of HIV-specific IL-21–producing Ag-experienced CD4$^+$ T cells are significantly reduced ($p < 0.01$) compared with HCMV-specific cells in HIV-infected individuals. However, we did not observe a significant difference between mean percentages of HIV-specific and HCMV-specific IFN-γ–producing cells in HIV-infected individuals. Only one subset of HCMV-specific Ag-experienced CD4$^+$ T cells (CD27$^-$CD45RA$^-$CCR7$^-$) were significantly reduced ($p < 0.01$) in HIV-infected individuals, compared with the same subset in healthy donors.

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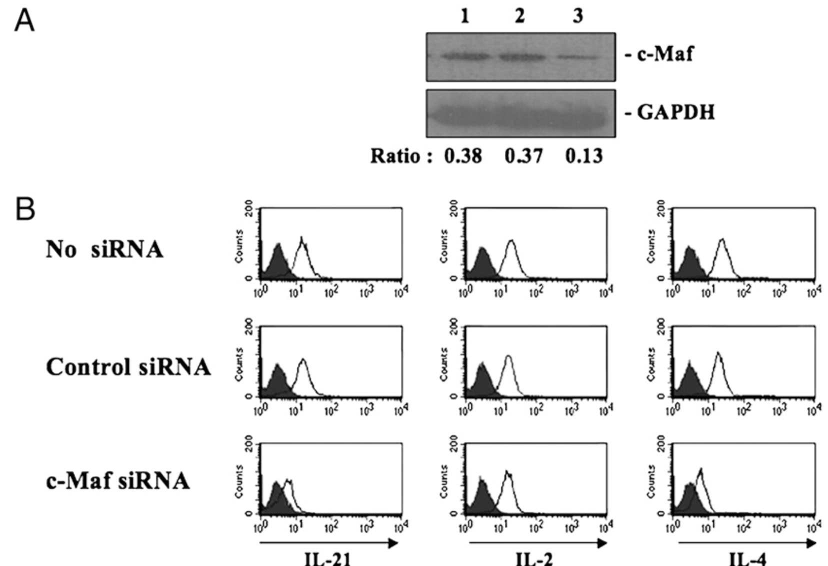


**FIGURE 7.** Inhibition of c-Maf expression in human CD4$^+$ T cells downregulates IL-21 expression. CD4$^+$ T cells were isolated from the PBMCs of an HIV-seronegative healthy donor, using a kit. The cells were transfected with c-Maf–specific or control siRNA duplexes. Posttransfection, the cells were cultured in vitro with ionomycin (1 μg/ml). After 24 h, the cells were treated for 4 h with brefeldin A, washed with PBS, and divided into three aliquots. Each aliquot was stained intracellularly for IL-21, IL-2 (negative control), or IL-4 (a positive control). *A*, Expression of c-Maf and GAPDH by Western blots in untransfected CD4$^+$ T cells (*lane 1*), the control siRNA-transfected cells (*lane 2*), and c-Maf siRNA-transfected cells (*lane 3*). *B*, Expression of intracellular IL-21, IL-2, or IL-4 in the transfected cells. The shaded area in each panel indicates staining for an isotype-matched control Ab. Note that a diminished expression of c-Maf results in a decreased expression of IL-21 and of another c-Maf–regulated cytokine, IL-4, but not of IL-2, which is not regulated by c-Maf.

Table II. *Cytokine production by HCMV- and HIV-1–specific Ag-experienced CD4+ T cells in viremic patients*

| | | Ag-Specific CD4+ T Cell Subsets | | | | | |
|---|---|---|---|---|---|---|---|
| | | CD27+CD45RA-CCR7+ | | CD27+CD45RA-CCR7- | | CD27-CD45RA-CCR7- | |
| Donors | Stimulation | IFN-γ+ | IL-21+ | IFN-γ+ | IL-21+ | IFN-γ+ | IL-21+ |
| Healthy | PBS | 0.18 ± 0.05 | 0.16 ± 0.05 | 0.19 ± 0.05 | 0.17 ± 0.05 | 0.21 ± 0.11 | 0.18 ± 0.05 |
| | CMV | 0.65 ± 0.12 | 0.52 ± 0.08 | 0.66 ± 0.18 | 0.48 ± 0.16 | 0.76 ± 0.12 | 0.52 ± 0.08 |
| | HIV-1 | — | — | — | — | — | — |
| Patients | PBS | 0.21 ± 0.01 | 0.18 ± 0.06 | 0.22 ± 0.01 | 0.16 ± 0.04 | 0.17 ± 0.05 | 0.17 ± 0.05 |
| | CMV | 0.48 ± 0.13 | 0.62 ± 0.18 | 0.54 ± 0.11 | 0.59 ± 0.09 | 0.58 ± 0.08 | 0.58 ± 0.07 |
| | HIV-1 | 0.67 ± 0.16 | 0.22 ± 0.08 | 0.58 ± 0.08 | 0.32 ± 0.11 | 0.63 ± 0.16 | 0.18 ± 0.08 |

Percentages of the indicated producing cells of three different subsets of Ag-experienced CD4+ T cells were determined 10 h after stimulation of patients' PBMCs with PBS, HCMV lysate, or HIV-1 lysate. The table shows mean ±SD of percentages of the cells from five healthy and five HIV-infected HAART-naive viremic donors.

### *IL-21 increases survival of CD4+ T cells*

Studies have shown that members of the γ-chain–using cytokine family (like IL-7 and IL-15) play a crucial role in the survival of T cells (33, 34). Furthermore, it is also known that circulating lymphocytes (including both CD4+ T cells and CD8+ T cells) from HIV-infected individuals undergo accelerated spontaneous apoptosis, compared with cells from HIV-seronegative healthy individuals, when these cells are cultured ex vivo under suboptimal conditions (e.g., reduced serum concentrations) (33–35). Therefore, we sought to investigate whether IL-21 could promote CD4+ T cell survival in HIV-infected individuals. For this purpose, PBMCs from healthy and HIV-infected patients were isolated and cultured ex vivo in the medium RPMI 1640 with 1% FCS with and without IL-21. After 72 h, the cells were harvested and stained for CD3, CD4, and 7-AAD. The percentages of dead (positive for 7-AAD) CD3+CD4+ cells were determined by flow cytometry. Fig. 8*B* shows cell death in CD3+CD4+ T cells with and without IL-21 addition in three healthy and three HIV-infected donors. The addition of IL-21 reduced cell death in CD3+CD4+ T cells in all healthy and HIV-infected donors. The average percentages of live CD4+ T cells with and without the cytokine addition for healthy and HIV-infected donors are shown in Fig. 8*C*. It is noteworthy that IL-21 restores the percentage of live CD4+ T cells comparable to that seen in IL-21–treated PBMCs obtained from HIV-seronegative healthy donors. The cytokine increased the percentages of live CD4+ T cells by 2.5-fold in PBMC cultures from healthy donors, whereas the increase was ~6.0-fold in PBMC cultures from HIV-infected donors. Taken together, these data show that IL-21 exerts a prosurvival effect on CD4+ T cells from both healthy and HIV-infected patients.

### *Expression of IL-21R increases on CD4+ T cells in HIV-infected individuals*

Decreased production of a cytokine often results in increased expression of its receptor on target cells. Therefore, we investigated whether a decreased production of IL-21 in HIV-infected individuals affects the expression of the cytokine receptor on CD4+ T cells. It is noteworthy that CD4+ T cells not only are major producers of the cytokine but also are its major target cells in the body. We compared the expression of the IL-21R on PBMCs obtained from HIV-infected and control subjects by flow cytometry after staining them for different fluorochrome-conjugated Abs against CD3, CD4, and IL-21Rα-chain. As shown in Fig. 9, CD3+CD4+ T cells from HIV-infected individuals demonstrate increased expression of the receptor, compared with cells from HIV-seronegative healthy individuals. Expression is significantly higher ($p < 0.01$) on CD4+ T cells from PI and CI patients than on cells from HIV-seronegative healthy controls.

### *HIV-infected individuals with higher serum levels of IL-21 have higher frequencies of HIV-specific CTL*

We measured the frequencies of HIV-specific CTL (CD3+CD8+ T cells) expressing IL-2, IFN-γ, and both together, as described in *Materials and Methods*. The HIV-specific CTL responses were clearly detectable in the PBMCs of all six HIV-infected individuals (Fig. 10). Frequencies of CTL responding to HIV-1 peptides in the control donors were almost similar to those seen in vehicle-treated PBMCs (data not shown). Furthermore, frequencies of the HIV-specific CTL producing single (IFN-γ or IL-2) as well as double cytokines (IFN-γ and IL-2) were significantly higher ($p < 0.05$) in HIV-infected patients with high levels of serum IL-21 than in HIV-infected individuals with low serum cytokine levels (Fig. 10*B*, 10*C*). These data suggest that IL-21 promotes higher frequencies, as well as functionality, of HIV-specific CTL in HIV-infected individuals.

## Discussion

The present study confirms earlier results from our laboratory (26), in which we showed decreased serum levels of IL-21 in HIV-infected individuals compared with those observed in HIV-seronegative healthy subjects. However, the earlier study was based only on a limited number of sera taken from a cross section of patients with chronic HIV infection. We extended our investigations to larger cohorts of patients, encompassing those with PI, CI, CI-H, and different categories (NP, SP, and EC) of LTNP. We also performed longitudinal studies on patients with PI who were or were not receiving HAART. Our results show for the first time that IL-21 production becomes compromised in early stages of the infection. Sera from all groups of HIV-infected patients showed decreased concentrations of the cytokine, compared with sera from HIV-seronegative healthy subjects. In this regard, only EC did not show a significant decrease in IL-21 levels in their sera. Longitudinal studies, as well as comparison between CI patients with and without HAART, revealed that HAART tended to increase serum concentrations of the cytokine, but only partially. Consistent with our results, partial restoration of other cytokines like IL-2 and IL-15 has also been observed in HIV-infected patients upon receiving HAART (36–39).

In all groups of HIV-infected individuals studied, we observed a strong correlation between CD4+ T cell counts and serum IL-21 concentrations. The correlation was also observed when serum IL-21 levels and CD4+ T cell counts of all HIV-infected patients were taken into consideration irrespective of clinical stage and HAART. These results strongly suggest that serum IL-21 levels could be used as surrogate markers for monitoring loss of CD4+ T cells, disease progression, and immune reconstitution in HIV-infected individuals.

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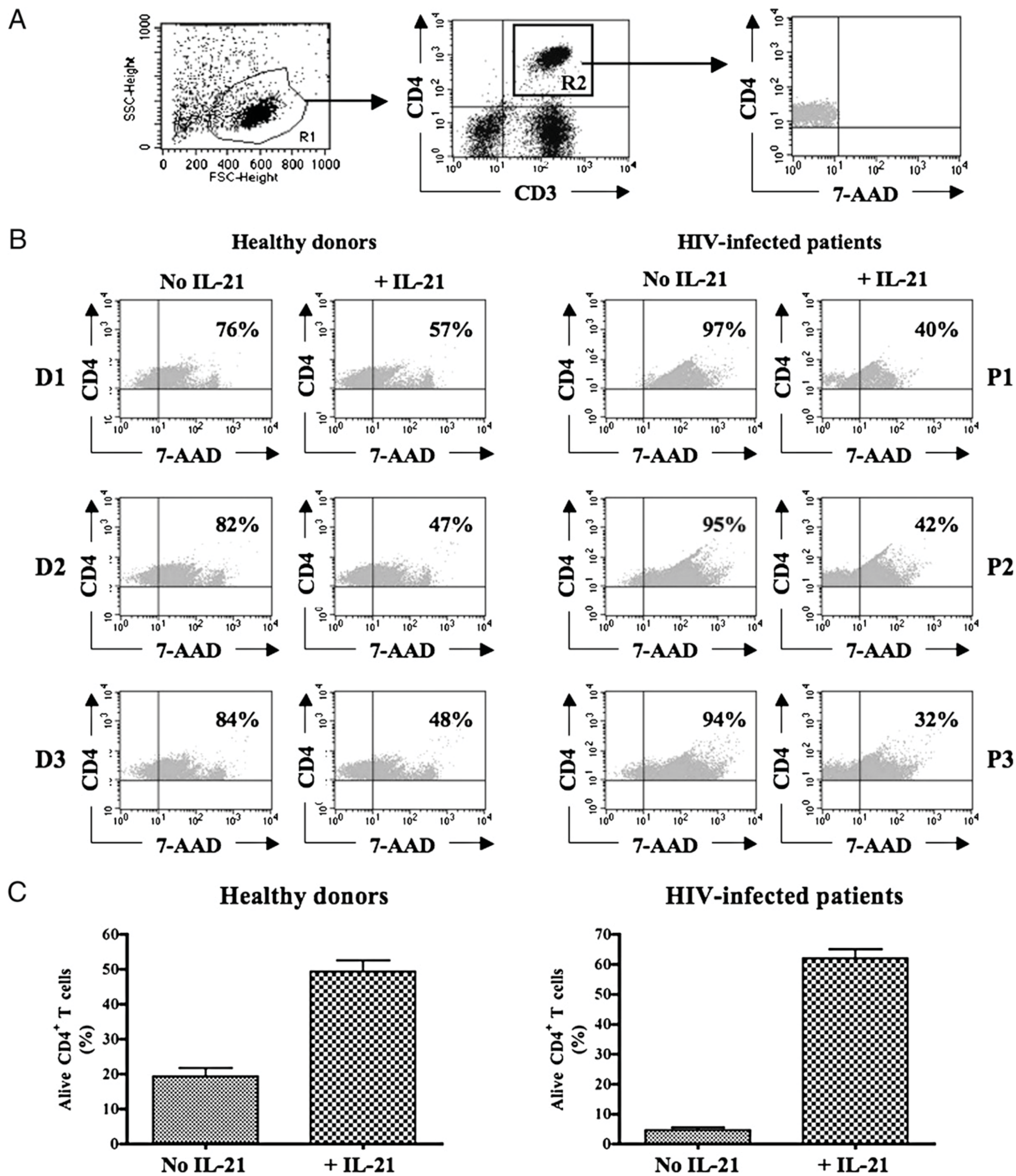


**FIGURE 8.** IL-21 is a prosurvival factor for $CD4^+$ T cells. *A*, PBMCs cultured in the medium supplemented with 10% FCS were stained with 7-AAD and used in a flow cytometer to set gates for live $CD3^+CD4^+$ T cells. *B*, PBMCs from three different healthy donors (D1, D2, and D3) and three HIV-infected viremic patients naive for HAART (P1, P2, and P3) were isolated and cultured for 72 h in RPMI 1640 supplemented with 1% FCS with or without IL-21 (15 μM). The cells were then harvested, stained with a mixture of Abs containing FITC-conjugated anti-CD4, PE-conjugated anti-CD3 Abs, and 7-AAD. The cells were washed, resuspended in PBS, and subjected to analysis by flow cytometry, counting 10,000 gated events. Cell death (positivity for 7-AAD) was measured by flow cytometry in $CD3^+CD4^+$ cells. The percentages of $CD3^+CD4^+$ T cells stained for 7-AAD are shown in each panel. *C*, Average percentages and SD of live $CD3^+CD4^+$ T cells with or without treatment with IL-21 are shown for HIV-infected and CONT donors.

These surrogate markers could be of great value when researchers and clinicians may have access only to serum samples from the patients. Furthermore, these results also indicate that depletion of $CD4^+$ T cells, which is a hallmark of AIDS, contributes toward decreased concentrations of the cytokine in the circulation of HIV-infected individuals.

In this paper, we have shown that $CD4^+$ T cells from HIV-infected individuals have a reduced ability to produce this cytokine, compared with their counterpart cells from healthy donors. In HIV-infected individuals, the frequencies of all major Ag-experienced HIV-specific IL-21–producing $CD4^+$ T cell subsets ($CD27^+CD45RA^-CCR7^+$, $CD27^+CD45RA^-CCR7^-$, and $CD27^-CD45RA^-CCR7^-$) were significantly decreased compared with the same subsets of HCMV specificity. Interestingly, the IFN-γ–producing HIV-specific Ag-experienced $CD4^+$ T cell subsets did not differ in frequency from their counterpart cells of HCMV specificity. These data suggest that HIV infection causes depletion of IL-21–producing Ag-experienced cells of only HIV specificity.

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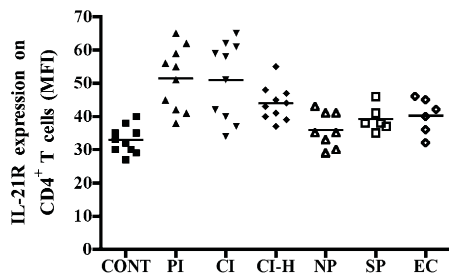


**FIGURE 9.** Expression of IL-21R on CD4+ T cells in HIV-infected individuals. PBMCs were stained with fluorochrome-conjugated anti-CD3, CD4, and IL-21Rα-chain Abs and analyzed by flow cytometry. Shown are mean fluorescence intensities (MFI) of IL-21R expression on CD3+CD4+ T cells from 10 individuals in each of the indicated donor groups: HIV-seronegative healthy CONT subjects and PI, CI, CI-H, NP, SP, EC patients. Vertical lines indicate mean MFI.

Although our data from in vitro studies on HIV-infected CD4+ T cells show that HIV infection does not affect IFN-γ production in infected cells, ex vivo studies on PBMCs from HIV-infected individuals reveal that the frequencies of these cytokine-producing HCMV-specific CD4+ T cells of the CD27−CD45RA−CCR7− phenotype are reduced in HIV-infected individuals compared with their counterpart cells from healthy donors. This difference may be attributed to the fact that in vivo many virus-induced host factors may also affect production of this and other cytokines. It is noteworthy that the CD27−CD45RA−CCR7− subset of CD4+ T cells is fully differentiated and produces maximum quantities of IFN-γ (40). Further studies are needed to determine what causes the reduced frequencies of IL-21–producing HIV-specific Ag-experienced CD4+ T cells in HIV-infected individuals.

We have shown in this study for the first time that in vitro infection of human CD4+ T cells reduces their ability to produce this cytokine. This may explain, at least in part, the compromised ability of CD4+ T cells from HIV-infected patients to produce this cytokine. However, it is noteworthy that not all CD4+ T cells in the circulation are infected with the virus (35). It is highly probable that one or more immunosuppressive cytokines (IL-10, TGF-β, etc.), which predominate in the circulation of HIV-infected patients (41, 42), may have downregulated the ability of CD4+ T cells to produce IL-21. Clearly further studies are needed to investigate these aspects of the cytokine's role in HIV infection.

It is well known that the transcription factor c-Maf plays a major part in the transcriptional activation of the *IL-21* gene (27). c-Maf knockout mice have drastically decreased levels of IL-21 in their circulation (27). c-Maf represents the cellular homolog of the viral oncogene *v-Maf* and was the first factor identified to be involved in the production of Th2-type cytokines. This factor regulates mainly cytokine production at the level of transcription. Enhanced production of c-Maf leads to an increase in IL-21 in CD4+ T cells (27, 32). Therefore, we sought to determine whether HIV-induced decreased production of this cytokine from human CD4+ T cells was due to any effect of the infection on this factor. The experiments indeed show that HIV infection of human PBMCs and CD4+ T cells in vitro results in reduced production of c-Maf. The downregulation occurs only in virus-infected cells, not in uninfected bystander cells. Our results are supported by our in vitro studies, in which a downregulation of c-Maf expression by siRNA caused a downregulation of IL-21 as well as another cytokine, IL-4, which is known to be positively regulated by the transcription factor (32). Interestingly, viral replication was needed for a reduced expression of IL-21 as well as c-Maf (Fig. 6). This is supported by our results that heat- and UV-inactivated HIV-1 preparations did not reduce IL-21 production from human PBMCs (data not shown). These results unravel the molecular mechanism underlying decreased production of this cytokine in HIV-infected individuals.

A decreased ex vivo survival of different lymphocytes from HIV-infected individuals has been well documented (33, 34, 43). CD4+ T cells are also targeted by the virus; therefore, depletion of these cells in the circulation of HIV-infected individuals is considered an AIDS-defining condition (25, 43). The restoration of these cells in HIV-infected AIDS patients is a real challenge. The use of HAART is highly effective in these patients in reducing viral load but is very slow in restoring CD4+ T cell counts to their physiological levels. This finding has led researchers to consider using immune-enhancing cytokines like IL-2, IL-15, IL-7 as therapeutic agents (17, 44–47). In this regard, our in vitro results demonstrate that IL-21 has the ability to prevent cell death in CD4+ T cells, and in ex vivo cultures it maintains the numbers of live cells to the levels seen in cultures of HIV-seronegative healthy donors (Fig. 8*B*). The prosurvival effects of the cytokine for CD4+ and CD8+ T cells have been demonstrated earlier (48). These workers showed that IL-21 activates PI-3K, increases Bcl-2 expression, and prevents decrease in cell size and spontaneous cell death in human T cells when cultured ex vivo. Our results demonstrate that the prosurvival effects of the cytokine on CD4+ T cells from HIV-infected individuals are greater than those on cells from HIV-seronegative healthy subjects. The cytokine induces a larger (6-fold) increase in the number of live CD4+ T cells from HIV-infected patients than that seen (2.5-fold) in HIV-seronegative healthy donor cultures. At least in part, this could be due to upregulated expression of the cytokine receptor on CD4+ T cells in HIV-infected individuals. These results suggest that decreased ex vivo survival of CD4+ T cells from HIV-infected patients may be a consequence of their decreased production of this cytokine. The cytokine may represent an appropriate immunotherapeutic tool for restoring survival of CD4+ T cells and other lymphocytes, hence enhancing their immune reconstitution in HIV-infected patients.

Studies have shown that IL-21 has many immune-enhancing effects. It increases the cytotoxic potential of CD8+ effector T cells by inducing perforin expression. The cytokine also activates NK cells and increases their degranulation ability (49, 50). Lower levels of the cytokine in HIV-infected individuals could result in compromised effector functions of these immune cells. Indeed, a decreased expression of perforin in these cells in HIV-infected individuals has been described (40). Further studies are needed to appreciate and understand the full spectrum of effects exerted by reduced production of this cytokine in HIV-infected individuals. Reduced levels of the cytokine may adversely affect survival, as well as the proliferation and functions of other immune cells such as CD8+ T cells and NK cells. Indeed, we have shown in this paper that increased serum levels of IL-21 are associated with higher frequencies and better functioning of HIV-specific CTL in HIV-infected individuals. Thus, a deficiency of the cytokine may adversely affect the ability of an individual to control the infection. Recent in vivo studies in mice have clearly demonstrated the indispensability of IL-21 in controlling chronic viral infections (14–16). A compromised production of IL-21 may be at least one reason why humans cannot efficiently control HIV infection.

Finally, it may be worth mentioning that IL-21, compared with other cytokines, is well tolerated by humans and is currently being tested for immunotherapy against melanoma and renal cell carcinoma in clinical trials (51, 52). Our studies suggest that it should be considered an immunotherapeutic tool in HIV-infected individuals.

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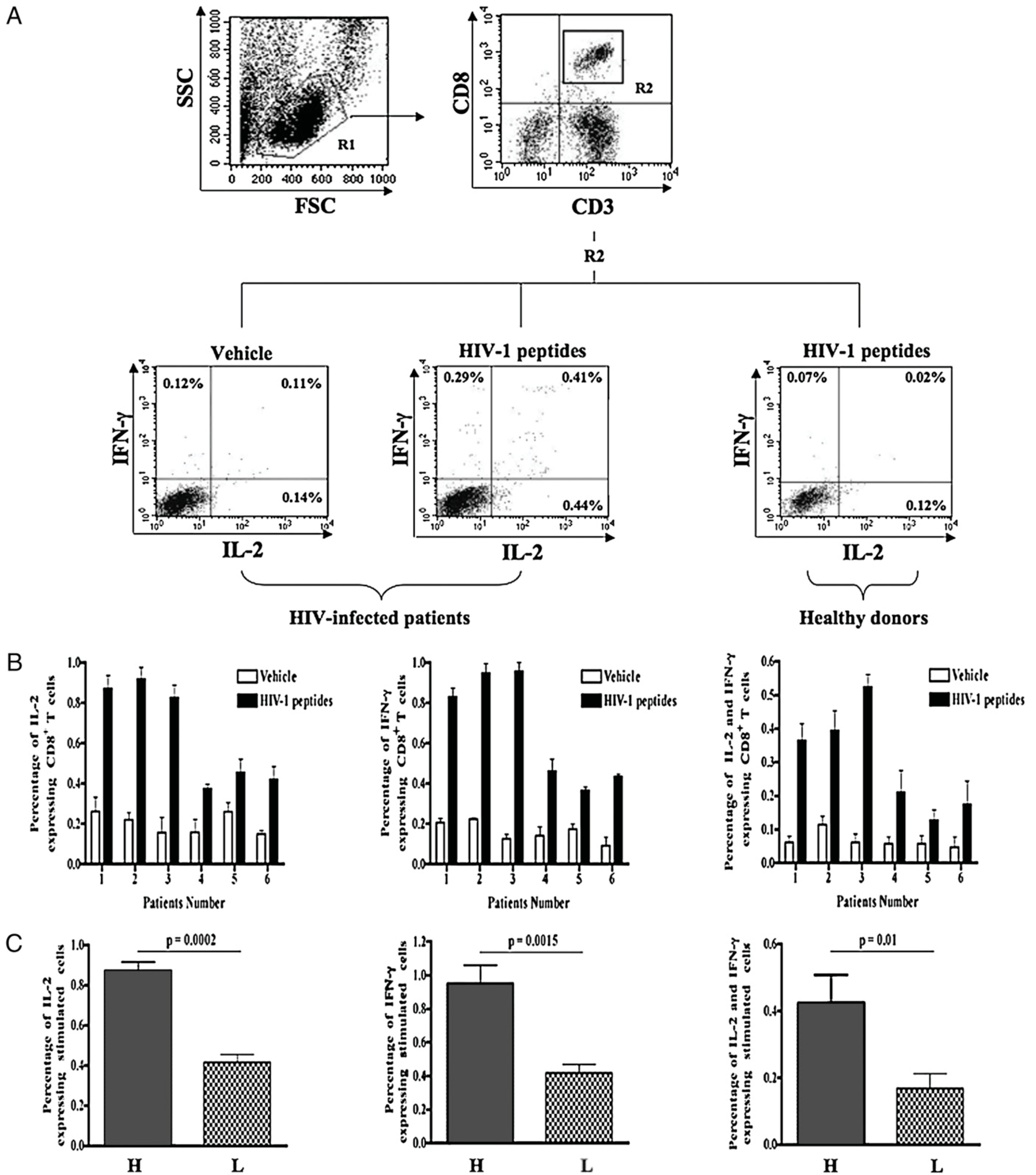


**FIGURE 10.** HIV-infected individuals with higher serum levels of IL-21 have higher frequencies of HIV-specific CTL. Ten million PBMCs from each HIV-infected or CONT subject were incubated for 10 h in the culture medium containing a pool of 20-mer overlapping peptides corresponding to the HxB2 Gag protein. Postincubation, the cells were stained with FITC-conjugated anti-CD8 and PE-Cy7–conjugated anti-CD3 Abs. The cells were also stained intracellularly with APC-conjugated anti–IL-2 and PE-conjugated IFN-γ Abs, and analyzed by flow cytometry. *A*, Gating of live cells for $CD3^{+}CD8^{+}$ T cells and their analyses for the intracellular expression of IL-2 and IFN-γ are shown. The panels show flow cytometric analysis for a typical HIV-infected individual and a typical CONT donor with HIV-1 peptide stimulation. *B*, Frequencies of HIV-specific CTL producing IL-2, IFN-γ, and both IL-2 and IFN-γ in response to HIV-1 peptides or a vehicle for HIV-infected individuals with high IL-21 serum levels (patient 1, 2, and 3 with 155, 210, and 189 pg/ml of IL-21, respectively) and low IL-21 serum levels (patient 4, 5, and 6 with 31, 18, and 29 pg/ml of IL-21, respectively). *C*, Comparisons are shown between HIV-infected individuals with low (L) levels of serum IL-21 and those with high (H) levels with regard to frequencies of different cytokine-producing, HIV-specific $CD8^{+}$ T cells in response to HIV-1 peptides.

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## Disclosures

The authors have no financial conflicts of interest.

## References

1. Parrish-Novak, J., S. R. Dillon, A. Nelson, A. Hammond, C. Sprecher, J. A. Gross, J. Johnston, K. Madden, W. Xu, J. West, et al. 2000. Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. *Nature* 408: 57–63.
2. Spolski, R., and W. J. Leonard. 2008. Interleukin-21: basic biology and implications for cancer and autoimmunity. *Annu. Rev. Immunol.* 26: 57–79.
3. Strengell, M., S. Matikainen, J. Sirén, A. Lehtonen, D. Foster, I. Julkunen, and T. Sareneva. 2003. IL-21 in synergy with IL-15 or IL-18 enhances IFN-γ production in human NK and T cells. *J. Immunol.* 170: 5464–5469.
4. Zeng, R., R. Spolski, S. E. Finkelstein, S. Oh, P. E. Kovanen, C. S. Hinrichs, C. A. Pise-Masison, M. F. Radonovich, J. N. Brady, N. P. Restifo, et al. 2005. Synergy of IL-21 and IL-15 in regulating T cell expansion and function. *J. Exp. Med.* 201: 139–148.
5. Wei, L., A. Laurence, K. M. Elias, and J. J. O'Shea. 2007. IL-21 is produced by Th17 cells and drives IL-17 production in a STAT3-dependent manner. *J. Biol. Chem.* 282: 34605–34610.
6. Yang, L., D. E. Anderson, C. Baecher-Allan, W. D. Hastings, E. Bettelli, M. Oukka, V. K. Kuchroo, and D. A. Hafler. 2008. IL-21 and TGF-β are required for differentiation of human T(H)17 cells. *Nature* 454: 350–352.
7. Korn, T., E. Bettelli, M. Oukka, and V. K. Kuchroo. 2009. IL-17 and Th17 Cells. *Annu. Rev. Immunol.* 27: 485–517.
8. Peluso, I., M. C. Fantini, D. Fina, R. Caruso, M. Boirivant, T. T. MacDonald, F. Pallone, and G. Monteleone. 2007. IL-21 counteracts the regulatory T cell-mediated suppression of human CD4+ T lymphocytes. *J. Immunol.* 178: 732–739.
9. Li, Y., and C. Yee. 2008. IL-21 mediated Foxp3 suppression leads to enhanced generation of antigen-specific CD8+ cytotoxic T lymphocytes. *Blood* 111: 229–235.
10. Ettinger, R., G. P. Sims, A.-M. Fairhurst, R. Robbins, Y. S. da Silva, R. Spolski, W. J. Leonard, and P. E. Lipsky. 2005. IL-21 induces differentiation of human naive and memory B cells into antibody-secreting plasma cells. *J. Immunol.* 175: 7867–7879.
11. Ettinger, R., G. P. Sims, R. Robbins, D. Withers, R. T. Fischer, A. C. Grammer, S. Kuchen, and P. E. Lipsky. 2007. IL-21 and BAFF/BLyS synergize in stimulating plasma cell differentiation from a unique population of human splenic memory B cells. *J. Immunol.* 178: 2872–2882.
12. Brandt, K., S. Bulfone-Paus, D. C. Foster, and R. Rückert. 2003. Interleukin-21 inhibits dendritic cell activation and maturation. *Blood* 102: 4090–4098.
13. Pelletier, M., A. Bouchard, and D. Girard. 2004. In vivo and in vitro roles of IL-21 in inflammation. *J. Immunol.* 173: 7521–7530.
14. Elsaesser, H., K. Sauer, and D. G. Brooks. 2009. IL-21 is required to control chronic viral infection. *Science* 324:1569–1572.
15. Frohlich, A., J. Kisielow, I. Schmitz, S. Freigang, A. T. Shamshiev, J. Weber, B. J. Marsland, A. Oxenius, and M. Kopf. 2009. IL-21R on T cells is critical for sustained functionality and control of chronic viral infection. *Science* 324: 1576–1580.
16. Yi, J. S., M. Du, and A. J. Zajac. 2009. A vital role for interleukin-21 in the control of a chronic viral infection. *Science* 324: 1572–1576.
17. Pahwa, S. 2007. Role of common γ chain utilizing cytokines for immune reconstitution in HIV infection. *Immunol. Res.* 38: 373–386.
18. Shearer, G. M. 1998. HIV-induced immunopathogenesis. *Immunity* 9: 587–593.
19. Sirskyj, D., J. Thèze, A. Kumar, and M. Kryworuchko. 2008. Disruption of the γ c cytokine network in T cells during HIV infection. *Cytokine* 43: 1–14.
20. Onoda, T., M. Rahman, H. Nara, A. Araki, K. Makabe, K. Tsumoto, I. Kumagai, T. Kudo, N. Ishii, N. Tanaka, et al. 2007. Human CD4+ central and effector memory T cells produce IL-21: effect on cytokine-driven proliferation of CD4+ T cell subsets. *Int. Immunol.* 19: 1191–1199.
21. Suto, A., D. Kashiwakuma, S.-i. Kagami, K. Hirose, N. Watanabe, K. Yokote, Y. Saito, T. Nakayama, M. J. Grusby, I. Iwamoto, and H. Nakajima. 2008. Development and characterization of IL-21-producing CD4+ T cells. *J. Exp. Med.* 205: 1369–1379.
22. Mohri, H., A. S. Perelson, K. Tung, R. M. Ribeiro, B. Ramratnam, M. Markowitz, R. Kost, A. Hurley, L. Weinberger, D. Cesar, et al. 2001. Increased turnover of T lymphocytes in HIV-1 infection and its reduction by antiretroviral therapy. *J. Exp. Med.* 194: 1277–1287.
23. Guadalupe, M., E. Reay, S. Sankaran, T. Prindiville, J. Flamm, A. McNeil, and S. Dandekar. 2003. Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. *J. Virol.* 77: 11708–11717.
24. Pantaleo, G., C. Graziosi, J. F. Demarest, L. Butini, M. Montroni, C. H. Fox, J. M. Orenstein, D. P. Kotler, and A. S. Fauci. 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature* 362: 355–358.
25. Pantaleo, G., C. Graziosi, and A. S. Fauci. 1993. The role of lymphoid organs in the pathogenesis of HIV infection. *Semin. Immunol.* 5: 157–163.
26. Iannello, A., C. Tremblay, J. P. Routy, M. R. Boulassel, E. Toma, and A. Ahmad. 2008. Decreased levels of circulating IL-21 in HIV-infected AIDS patients: correlation with CD4+ T-cell counts. *Viral Immunol.* 21: 385–388.
27. Bauquet, A. T., H. Jin, A. M. Paterson, M. Mitsdoerffer, I. C. Ho, A. H. Sharpe, and V. K. Kuchroo. 2009. The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and TH-17 cells. *Nat. Immunol.* 10: 167–175.
28. Iannello, A., S. Samarani, O. Debbeche, R. Ahmad, M.-R. Boulassel, C. Tremblay, E. Toma, J.-P. Routy, and A. Ahmad. 2009. Potential role of interleukin-18 in the immunopathogenesis of AIDS: involvement in fratricidal killing of NK cells. *J. Virol.* 83: 5999–6010.
29. Ahmad, R., S. T. A. Sindhu, E. Toma, R. Morisset, and A. Ahmad. 2002. Elevated levels of circulating interleukin-18 in human immunodeficiency virus-infected individuals: role of peripheral blood mononuclear cells and implications for AIDS pathogenesis. *J. Virol.* 76: 12448–12456.
30. Appay, V., R. van Lier, F. Sallusto, and M. Roederer. 2008. Phenotype and function of human T lymphocyte subsets: consensus and issues. *Cytometry A* 73: 975–983.
31. Coates, J. A., N. Cammack, H. J. Jenkinson, A. J. Jowett, M. I. Jowett, B. A. Pearson, C. R. Penn, P. L. Rouse, K. C. Viner, and J. M. Cameron. 1992. (-)-2′-deoxy-3′-thiacytidine is a potent, highly selective inhibitor of human immunodeficiency virus type 1 and type 2 replication in vitro. *Antimicrob. Agents Chemother.* 36: 733–739.
32. Ho, I. C., D. Lo, and L. H. Glimcher. 1998. c-maf promotes T helper cell type 2 (Th2) and attenuates Th1 differentiation by both interleukin 4-dependent and -independent mechanisms. *J. Exp. Med.* 188: 1859–1866.
33. Vassena, L., M. Proschan, A. S. Fauci, and P. Lusso. 2007. Interleukin 7 reduces the levels of spontaneous apoptosis in CD4+ and CD8+ T cells from HIV-1-infected individuals. *Proc. Natl. Acad. Sci. USA* 104: 2355–2360.
34. Naora, H., and M. Gougeon. 1999. Interleukin-15 is a potent survival factor in the prevention of spontaneous but not CD95-induced apoptosis in CD4 and CD8 T lymphocytes of HIV-infected individuals. Correlation with its ability to increase BCL-2 expression. *Cell Death Differ.* 6: 1002–1011.
35. Moanna, A., R. Dunham, M. Paiardini, and G. Silvestri. 2005. CD4+ T-cell depletion in HIV infection: killed by friendly fire? *Curr. HIV/AIDS Rep.* 2: 16–23.
36. Brazille, P., N. Dereuddre-Bosquet, C. Leport, P. Clayette, O. Boyer, J. L. Vildé, D. Dormont, and O. Benveniste. 2003. Decreases in plasma TNF-α level and IFN-γ mRNA level in peripheral blood mononuclear cells (PBMC) and an increase in IL-2 mRNA level in PBMC are associated with effective highly active antiretroviral therapy in HIV-infected patients. *Clin. Exp. Immunol.* 131: 304–311.
37. Clerici, M., C. Balotta, L. Meroni, E. Ferrario, C. Riva, D. Trabattoni, A. Ridolfo, M. Villa, G. M. Shearer, M. Moroni, and M. Galli. 1996. Type 1 cytokine production and low prevalence of viral isolation correlate with long-term nonprogression in HIV infection. *AIDS Res. Hum. Retroviruses* 12: 1053–1061.
38. d'Ettorre, G., G. Forcina, M. Andreotti, L. Sarmati, L. Palmisano, M. Andreoni, S. Vella, C. M. Mastroianni, and V. Vullo. 2004. Interleukin-15 production by monocyte-derived dendritic cells and T cell proliferation in HIV-infected patients with discordant response to highly active antiretroviral therapy. *Clin. Exp. Immunol.* 135: 280–285.
39. Isgrò, A., A. Aiuti, I. Mezzaroma, M. Addesso, E. Riva, A. Giovannetti, F. Mazzetta, C. Alario, A. Mazzone, L. Ruco, and F. Aiuti. 2002. Improvement of interleukin 2 production, clonogenic capability and restoration of stromal cell function in human immunodeficiency virus-type-1 patients after highly active antiretroviral therapy. *Br. J. Haematol.* 118: 864–874.
40. Appay, V., D. F. Nixon, S. M. Donahoe, G. M. A. Gillespie, T. Dong, A. King, G. S. Ogg, H. M. L. Spiegel, C. Conlon, C. A. Spina, et al. 2000. HIV-specific CD8(+) T cells produce antiviral cytokines but are impaired in cytolytic function. *J. Exp. Med.* 192: 63–75.
41. Stylianou, E., P. Aukrust, D. Kvale, F. Müller, and S. S. Frøland. 1999. IL-10 in HIV infection: increasing serum IL-10 levels with disease progression—down-regulatory effect of potent anti-retroviral therapy. *Clin. Exp. Immunol.* 116: 115–120.
42. Wiercińska-Drapalo, A., R. Flisiak, J. Jaroszewicz, and D. Prokopowicz. 2004. Increased plasma transforming growth factor-beta1 is associated with disease progression in HIV-1-infected patients. *Viral Immunol.* 17: 109–113.
43. Estaquier, J., T. Idziorek, F. de Bels, F. Barré-Sinoussi, B. Hurtrel, A. M. Aubertin, A. Venet, M. Mehtali, E. Muchmore, P. Michel, et al. 1994. Programmed cell death and AIDS: significance of T-cell apoptosis in pathogenic and nonpathogenic primate lentiviral infections. *Proc. Natl. Acad. Sci. USA* 91: 9431–9435.
44. Aiuti, F., and I. Mezzaroma. 2006. Failure to reconstitute CD4+ T-cells despite suppression of HIV replication under HAART. *AIDS Rev.* 8: 88–97.
45. Imami, N., G. Hardy, A. Pires, C. Burton, J. Pido-Lopez, C. Mela, and F. Gotch. 2002. Immune reconstitution in HIV-1-infected patients. *Curr. Opin. Investig. Drugs* 3: 1138–1145.
46. Levy, Y., C. Lacabaratz, L. Weiss, J. P. Viard, C. Goujard, J. D. Lelièvre, F. Boué, J. M. Molina, C. Rouzioux, V. Avettand-Fénoêl, et al. 2009. Enhanced T cell recovery in HIV-1-infected adults through IL-7 treatment. *J. Clin. Invest.* 119: 997–1007.
47. Sereti, I., R. M. Dunham, J. Spritzler, E. Aga, M. A. Proschan, K. Medvik, C. A. Battaglia, A. L. Landay, S. Pahwa, M. A. Fischl, et al. 2009. IL-7 administration drives T cell-cycle entry and expansion in HIV-1 infection. *Blood.* 113: 6304–6314.
48. Ostiguy, V., E.-L. Allard, M. Marquis, J. Leignadier, and N. Labrecque. 2007. IL-21 promotes T lymphocyte survival by activating the phosphatidylinositol-3 kinase signaling cascade. *J. Leukoc. Biol.* 82: 645–656.
49. White, L., S. Krishnan, N. Strbo, H. Liu, M. A. Kolber, M. G. Lichtenheld, R. N. Pahwa, and S. Pahwa. 2007. Differential effects of IL-21 and IL-15 on perforin expression, lysosomal degranulation, and proliferation in CD8 T cells of patients with human immunodeficiency virus-1 (HIV). *Blood* 109: 3873–3880.
50. Strbo, N., L. de Armas, H. Liu, M. A. Kolber, M. Lichtenheld, and S. Pahwa. 2008. IL-21 augments natural killer effector functions in chronically HIV-infected individuals. *AIDS* 22: 1551–1560.
51. Andorsky, D. J., and J. M. Timmerman. 2008. Interleukin-21: biology and application to cancer therapy. *Expert Opin. Biol. Ther.* 8: 1295–1307.
52. Skak, K., M. Kragh, D. Hausman, M. J. Smyth, and P. V. Sivakumar. 2008. Interleukin 21: combination strategies for cancer therapy. *Nat. Rev. Drug Discov.* 7: 231–240.

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## 12-2 Effet de l'IL-21 sur les cellules Natural Killer et la réplication du VIH-1

Les résultats obtenus lors de cette étude concernant l'effet de l'IL-21 sur la fonctionnalité des cellules NK ainsi que l'impact de cette cytokine sur la réplication virale *in vitro* sont présentés sous le format d'un article de recherche. Cet article a été soumis à un processus de révision par les pairs avant sa publication.

**Article 10 : Iannello A**, Boulassel MR, Samarani S, Tremblay C, Toma E, Routy JP, and Ahmad A. IL-21 enhances NK cell functions and survival in healthy and HIV-infected patients with minimal stimulation of viral replication. 2010. ***Journal of Leukocyte Biology*** **87(5):857-67**.

**Résumé :** Lors de cette étude, nous avons investigué comment l'IL-21 exogène augmentait la réponse immunitaire médiée par les cellules NK chez les patients infectés. Nous avons démontré que l'IL-21R était exprimé de façon égale et constitutive sur tous les sous-types majeurs de cellules NK chez les personnes infectées par le VIH-1 ainsi que chez les personnes saines. L'IL-21 active les protéines STAT-3, MAPK et Akt afin d'augmenter les fonctions effectrices des cellules NK. Nous avons démontré que l'IL-21 augmentait l'expression des protéines Bcl-2 et Bcl-$X_L$ et que cette cytokine augmente la viabilité des cellules NK sans avoir un effet direct sur leur prolifération. Notre étude a démontré que l'IL-21 augmente l'ADCC spécifique au VIH-1, les fonctions sécrétrices et cytotoxiques, ainsi que la viabilité des cellules NK provenant de patients infectés virémiques et sans traitement anti-rétroviral. De plus, nos études ont démontré que l'IL-21 peut avoir ces effets sur les cellules NK sans stimuler la réplication du VIH-1. Nous avons en effet démontré que les cellules NK activées par l'IL-21 inhibent la réplication virale lors de co-cultures avec des cellules T $CD4^+$ infectées. Cette inhibition a lieu lors d'un contact entre la cellule NK et sa cellule cible, et non par la sécrétion de facteurs solubles.

# Article 10

**Iannello A**, Boulassel MR, Samarani S, Tremblay C, Toma E, Routy JP, and Ahmad A. IL-21 enhances NK cell functions and survival in healthy and HIV-infected patients with minimal stimulation of viral replication. 2010. ***Journal of Leukocyte Biology*** **87(5):857-67**.

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Brief Conclusive Report

# IL-21 enhances NK cell functions and survival in healthy and HIV-infected patients with minimal stimulation of viral replication

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## ABSTRACT

**IL-21 plays an important role in regulating immune response and controlling chronic viral infections. Recently, we reported its decreased serum concentrations and their immunological consequences in HIV-infected persons. In this study, we have investigated how exogenous IL-21 enhances NK cell responses in these persons. We show that the cytokine receptors are expressed equally on all NK cell subsets defined by expression of CD16 and CD56; the cytokine activates STAT-3, MAPK, and Akt to enhance NK cell functions; the STAT-3 activation plays a key role in constitutive and IL-21-mediated enhancement of NK cell functions; the cytokine increases expression of antiapoptotic proteins Bcl-2 and Bcl-$X_L$ and enhances viability of NK cells but has no effect on their proliferation; the cytokine enhances HIV-specific ADCC, secretory, and cytotoxic functions, as well as viability of NK cells from HIV-infected persons; it exerts its biological effects on NK cells with minimal stimulation of HIV-1 replication; and the cytokine-activated NK cells inhibit viral replication in cocultured, HIV-infected, autologous $CD4^+$ T cells in a perforin- and LFA-1-dependent manner. These data suggest that IL-21 may serve as a valuable therapeutic tool for enhancing NK cell responses and inhibiting viral replication in HIV-infected patients. *J. Leukoc. Biol.* 87: 857–867; 2010.**

## Introduction

IL-21 is a relatively recently discovered multifunctional and pleiotropic cytokine [1, 2]. It is a member of the common γc-using cytokine family that includes IL-2, IL-4, IL-7, IL-9, and IL-15. The cytokine is produced mainly by central and effector memory $CD4^+$ T cells of the TH1 and TH17 types but not by resting or activated, naïve $CD4^+$ T cells. In addition, follicular Th cells, thymic stromal cells, and NKT cells produce this cytokine [1, 3]. IL-21 exerts many immunoregulatory and immune-enhancing properties and acts as a key factor for controlling chronic viral infections in vivo [1, 4]. It induces maturation and differentiation of T, B, and NK cells and enhances their antiviral effector functions [1, 5]. Since its discovery in 2000, several studies have demonstrated that IL-21, alone and/or in combination with other cytokines such as IL-2, IFN-α, or IL-15, activates NK cells and enhances their proliferation, cytotoxicity, and secretion of IFN-γ via activating PI3K and STAT-3 [2, 6–9]. In fact, its anti-tumor effects have been ascribed to its activating effects on NK cells [10].

NK cells represent an important cellular component of innate immunity. They often kill virus-infected cells without prior sensitization and amplification directly as well as via ADCC (reviewed in ref. [11]). Relatively higher levels of activation of NK cells have been associated with protection from HIV-1 infection as well as with delayed progression to AIDS [11, 12]. Perturbations in NK cell activities and in different NK cell subsets have been observed in HIV-infected persons and have been implicated in the progression of AIDS (ref. [13]; reviewed in ref. [11]). The treatment of HIV-infected individuals with HAART is generally very effective in reducing viral loads to nondetectable levels in the infected patients. However, the treated patients are slow or are unable to regain their normal immune competence. For this reason, researchers are resorting to the use of various immune-enhancing cytokines, such as IL-2, IL-7, or IL-15, in these patients. Given that IL-21 possesses many immune-enhancing properties, and its production declines progressively with disease progression in HIV-infected persons [14, 15], it could be a potential therapeutic tool in this setting. However, only one study has so far examined the immune-enhancing effects of this cytokine on

Abbreviations: 7-AAD=7-amino-actinomycin, ADCC=antibody-dependent cell-mediated cytotoxicity, APC=allophycocyanin, γc=γ-chain, h=human, HAART=highly active anti-retroviral therapy, LTNP=long-term, nonprogressors, MFI=mean fluorescence intensity

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NK cells in the context of HIV-1 infection [16]. As our laboratory has since long been interested in studying NK cell functions in HIV-infected persons, we sought to investigate the immune-enhancing effects of the cytokine on NK cell functions in these patients. In addition to investigating the immune-enhancing effects of IL-21 on NK cells from HIV-infected individuals, we also determined the effect of this cytokine on HIV-1 replication. We show that all NK cell subsets express equivalent levels of IL-21R on their surface. It increases direct cytotoxic activity of NK cells as well as their ADCC effector functions against target cells expressing the viral envelope glycoprotein gp120. We also show here that IL-21 enhances NK cells survival in low-serum conditions, maintains physiological levels of Bcl-2 and Bcl-$X_L$ proteins, but promotes their proliferation only when combined with other cytokines. We have also reexamined IL-21-induced signaling pathways in human NK cells and linked them to distinct functions of these cells. The cytokine induces activation of STAT-3, ERK-1/ERK-2, p38, and Akt in human NK cells. Furthermore, we also show here that IL-21 increases ADCC, natural cytotoxicity, survival, and the ability to degranulate in NK cells from viremic, HIV-infected persons without stimulating HIV-1 replication. Interestingly, our in vitro studies show that the presence of IL-21 abrogates HIV-1 replication in the cocultures of NK cells with HIV-infected, autologous $CD4^+$ T cells. Collectively, these data suggest the potential usefulness of this cytokine as an immunotherapeutic tool for enhancing antiviral NK cell responses in HIV-infected persons.

## MATERIALS AND METHODS

### Cell culture, antibodies, and reagents

The cells were cultured in the medium RPMI 1640 (Gibco, Burlington, ON, Canada) containing 10% FBS, 2 mM L-glutamine, and antibiotics as described [15]. The NK92 cells (a human NK cell line) were maintained in α-MEM (Gibco) containing 12.5% FBS, 12.5% horse serum, and 100 U/ml IL-2. CEM cells expressing the HIV-1 gp120/41 (CEM-gp120/41) and control cells were generated in our laboratory as described earlier [17] and were maintained in culture medium containing neomycin (1 μg/ml; Gibco). Human NK and $CD4^+$ T cells were isolated from PBMC by negative selection using respective cell separation kits (Stem Cell Technology, BC, Canada). The purity of the isolated cells was verified and was always >97% for NK and $CD4^+$ T cells. rhIL-15 and rhIL-21 were purchased from BioSource (Camarillo, CA, USA), and IL-2 was from Roche (Mississauga, ON, Canada). The antibodies and pharmacological inhibitors used in this study were purchased from commercial sources, which are mentioned at relevant places in the text and figure legends.

### Study participants

In some experiments, we used PBMC from HIV-seronegative, healthy individuals and HIV-infected patients. Of these patients, five had primary infection (defined as being within 6 months following the date of infection and receiving no antiretroviral therapy or HAART; median±SD for CD4 counts: 413±133 cells/$mm^3$; median±SD for viral load: 158,500±6340 HIV-1 RNA copies/ml); 11 had chronic infection and were viremic (having infection for more than 6 months and naïve for HAART, median±SD for CD4 counts: 161±137 cells/$mm^3$; median±SD for viral load: 218,776±27,347 HIV-1 RNA copies/ml); five had chronic infection, were receiving HAART, and had undetectable plasma viral loads (median±SD for CD4 counts: 524±121 cells/$mm^3$; median±SD for viral load: 50±5 HIV-1 RNA copies/ml); and five were LTNP (infected for more than 7 years, not receiving HAART, and without having any AIDS-defining condition; median±SD for CD4 counts: 600±250 cells/$mm^3$; median±SD for viral load: 95±15 HIV-1 RNA copies/ml). All of the study participants volunteered for a one-time blood donation and gave written, informed consent. The Institutional Ethics Committee approved the study protocols.

### Expression of proteins

Western blots were used for detecting expression of different cellular proteins using antibodies specific for the protein or its activated form as described previously [15].

### Flow cytometry

Cells were stained for the molecules expressed on the surface or inside cells and analyzed by flow cytometry as described earlier [15].

### NK cell-mediated cytotoxicity and ADCC

They were determined with a standard 16-h $^{51}Cr$-release (Perkin Elmer, Boston, MA, USA) assay, as described by us earlier [17]. The procedure for the ADCC assay was the same as for NK cell cytotoxicity with two exceptions: First, CEM-gp120/41 cells were used as target cells, and second, 50 μl culture medium containing 5 μl sera from HIV/AIDS patients or healthy subjects was added to each well. After 16 h incubation at 37°C in a humidified 5% $CO_2$ atmosphere, the radioactivity released into the supernatants was counted by a γ-counter (Clini Gamma, LKB Wallace, Turku, Finland). The percentage lysis of the target cells was determined as described earlier [17].

### Cell proliferation

The cell proliferation was measured by $^3H$-thymidine (Amersham Biosciences, Quebec, Canada) uptake assay, as described [18].

### Statistical Analysis

Group means were compared using unpaired Student's *t*-test and ANOVA as described earlier [15].

## RESULTS AND DISCUSSION

### All subsets of NK cells constitutively express IL-21R

NK cells are known to be heterogeneous with respect to the expression of CD16 and CD56 markers. Although two major subsets of NK cells ($CD56^{dim}$ and $CD56^{bright}$) have been shown previously to respond to IL-21 and express equivalent levels of the cytokine receptor [19], it is not yet known if all subsets of NK cells defined by the expression of CD56 and CD16 markers express this receptor. Using flow cytometry, we analyzed the expression of the IL-21R α-chain on different subsets of NK cells. As shown in **Figure 1, A** and **B**, all subsets of NK cells ($CD56^{bright}CD16^+$, $CD56^{bright}CD16^-$, $CD56^{dim}CD16^+$, and $CD56^-CD16^+$) expressed equivalent levels of the cytokine receptor. Consequently, these data suggest that all NK cells are likely to respond to exogenous rhIL-21. Furthermore, we investigated whether the expression of the cytokine receptor remained intact on NK cells from HIV-infected individuals. For this purpose, we compared the expression of IL-21R on NK cell subsets from different categories of HIV-infected patients by flow cytometry with control subjects. The patient categories included five patients with primary infection, five with chronic infection, five with chronic infection receiving HAART, and five LTNP, as described in Materials and Methods. No statisti-

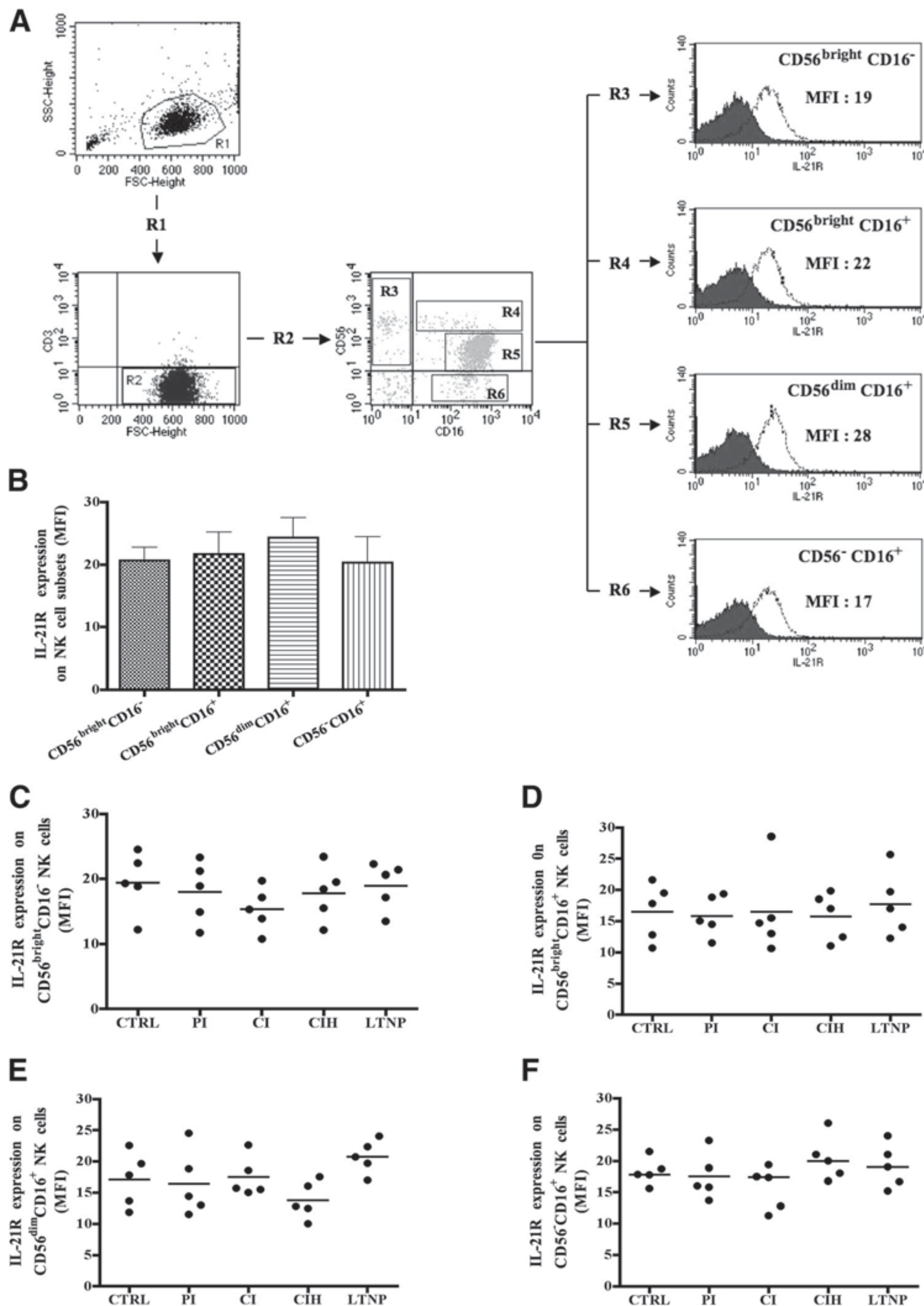


**Figure 1. IL-21R is constitutively expressed on all subsets of NK cells.** (A and B) Isolated NK cells from healthy donors were stained with APC-conjugated anti-CD56, PerCP-conjugated anti-CD3, PE-conjugated anti-IL-21R$\alpha$-chain, FITC-conjugated anti-CD16 antibodies, or with their respective isotypes as control. All antibodies were from eBioscience (San Diego, CA, USA). Alive NK cells were gated (R1) using forward- and side-scatter (FSC and SSC, respectively) profiles. From the R1 population, $CD3^-$ cells were gated (R2) and analyzed for the expression of CD56 and CD16. The $CD56^{bright}CD16^-$ (R3), $CD56^{bright}CD16^+$ (R4), $CD56^{dim}CD16^+$ (R5), and $CD56^-CD16^+$ (R6) populations were further gated and analyzed for the expression of their IL-21R$\alpha$-chain expression. The filled and open histograms represent staining with isotype and anti-IL-21R$\alpha$ antibodies, respectively. All four subsets of NK cells show equivalent expression (similar MFI) of the receptor chain. (B) Averages ± SD (MFI) obtained for all NK subsets from three different healthy donors. IL-21R expression on NK cell subsets in HIV-infected patients is shown in C–F. PBMC from five healthy subjects [control (CTRL)] as well as five HIV-infected patients belonging to different categories (PI=primary infection; CI=chronic infection and viremic; CIH=chronic infection and aviremic) were stained with FITC-conjugated anti-CD16, APC-conjugated anti-CD56, PE-Cy7-conjugated anti-CD3 (all from eBioscience), and PE-conjugated anti-IL-21R-$\alpha$ (BD Biosciences, Mississauga, ON, Canada) antibodies and respective control isotypes. The four different NK cell subsets, $CD56^{bright}CD16^-$ (C), $CD56^{bright}CD16^+$ (D), $CD56^{dim}CD16^+$ (E), and $CD56^-CD16^+$ (F) were further gated and analyzed for the expression of their IL-21R$\alpha$-chain expression. Note that the four NK cells subsets from healthy donors as well as HIV-infected patients show equivalent expression (similar MFI) of the receptor chain.

cally significant ($P$>0.05) differences in IL-21R expression were observed for all NK cell subsets between HIV-infected and healthy individuals (Fig. 1, C–F).

## IL-21 increases cytotoxicity and ADCC mediated by NK cells

We re-examined the effects of exogenous human IL-21 on NK cell functions in vitro. For this purpose, we purified NK cells from freshly isolated human PBMC and cultured them with or without IL-21 for 24 h. After 24 h, the cytotoxic activity of the NK cells against an established human T cell line, CEM, was determined in a standard 16-h $^{51}$Cr-release assay at different E:T ratios. In these experiments, IL-21 increased cytotoxic activity of NK cells equivalent to that induced by equimolar concentrations of IL-2 or IL-15. Furthermore, we also observed an additive effect of IL-21 with IL-2 and with IL-15 on the NK cell-mediated cytotoxicity (data not shown). These NK cell-mediated, cytotoxicity-enhancing effects of IL-21 are in agreement with those reported earlier [8, 16, 20].

IL-21 has been shown to enhance NK cell-mediated ADCC against antibody-coated target cells [21]. However, these studies were carried out in the context of breast cancer. We were

interested in knowing whether the cytokine will also enhance ADCC effector function of NK cells in the context of HIV infection. For this purpose, we used our gp120/41 expressing CEM cells in the presence of sera from HIV-infected persons known to have gp120/41-specific, ADCC-mediating antibodies [17]. As shown in **Figure 2A**, IL-21 treatment significantly enhanced ADCC mediated by the NK cells when they were incubated in the presence of sera from HIV-infected patients as compared with the untreated cells ($P<0.001$). As shown in Figure 2B, this increase was comparable to the one induced by IL-2 or by IL-15. No additive effects of IL-21 and IL-2 or of IL-21 and IL-15 were observed on the ADCC-mediated killing, probably as a result of high levels of background (i.e., the killing of the target cells in the presence of control serum). As NK cells mediate ADCC via CD16 (FcγRIIIa), which is a low-affinity receptor for the Fc region of IgG

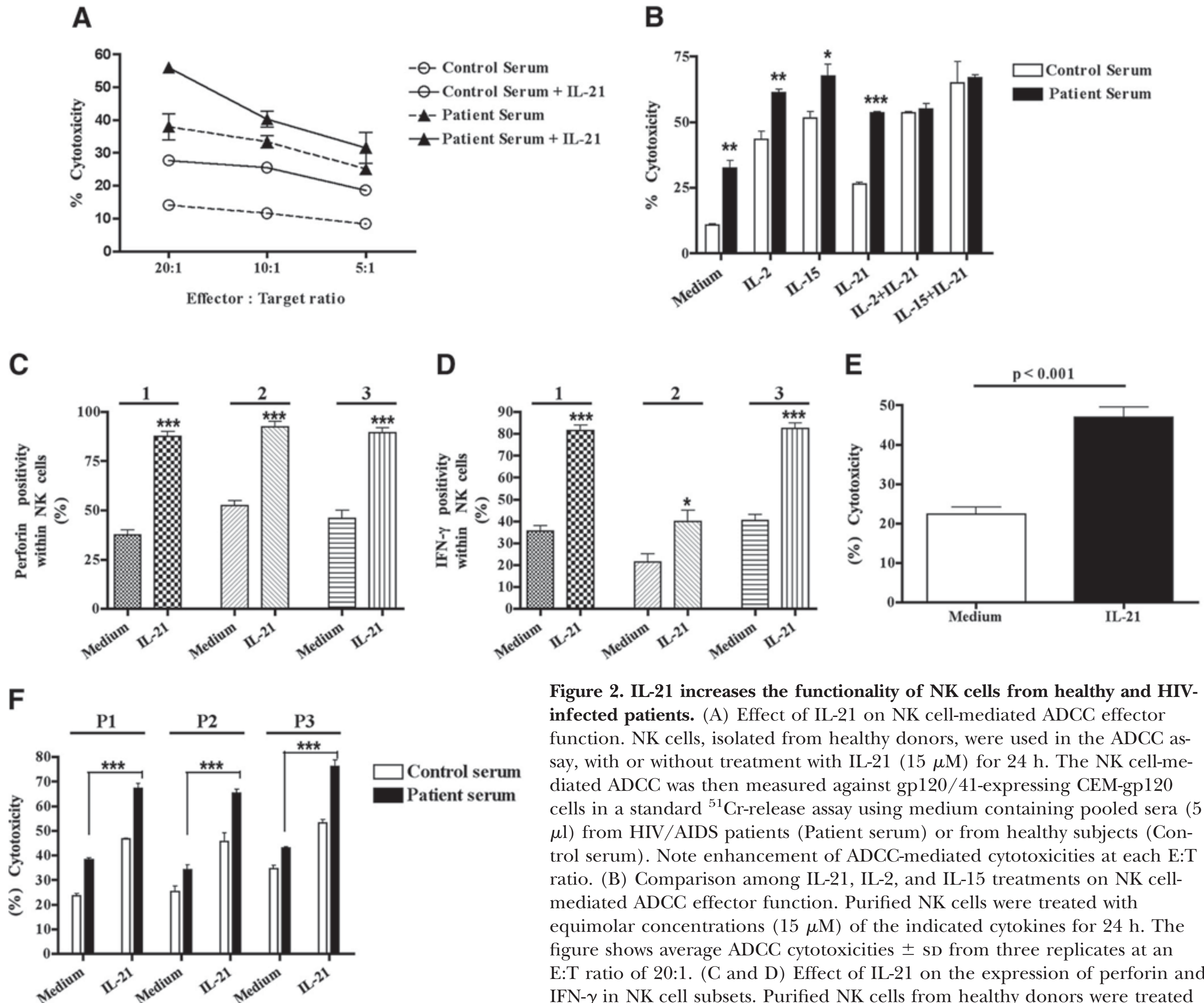


**Figure 2. IL-21 increases the functionality of NK cells from healthy and HIV-infected patients.** (A) Effect of IL-21 on NK cell-mediated ADCC effector function. NK cells, isolated from healthy donors, were used in the ADCC assay, with or without treatment with IL-21 (15 μM) for 24 h. The NK cell-mediated ADCC was then measured against gp120/41-expressing CEM-gp120 cells in a standard $^{51}$Cr-release assay using medium containing pooled sera (5 μl) from HIV/AIDS patients (Patient serum) or from healthy subjects (Control serum). Note enhancement of ADCC-mediated cytotoxicities at each E:T ratio. (B) Comparison among IL-21, IL-2, and IL-15 treatments on NK cell-mediated ADCC effector function. Purified NK cells were treated with equimolar concentrations (15 μM) of the indicated cytokines for 24 h. The figure shows average ADCC cytotoxicities ± SD from three replicates at an E:T ratio of 20:1. (C and D) Effect of IL-21 on the expression of perforin and IFN-γ in NK cell subsets. Purified NK cells from healthy donors were treated with 15 μM IL-21 for 24 h. Aliquots of the cells were stained extracellularly with APC-conjugated anti-CD56 and FITC-conjugated anti-CD16 and intracellularly with PE-conjugated anti-perforin or with PE-conjugated anti-IFN-γ (all antibodies from eBioscience). The $CD56^{bright}CD16^{+}$ (1) and $CD56^{dim}CD16^{+}$ (2) populations were further gated and analyzed for the expression of perforin (C) and IFN-γ (D). In separate experiments, NK92 cells (3) were also analyzed for expression of perforin (C) and IFN-γ (D). Similar results were obtained with three different donors. (E) Spontaneous cytotoxic activities of isolated NK cells obtained from six viremic, HAART-naïve, HIV-infected patients were determined against $^{51}$Cr-labeled CEM at E:T ratios of 20:1. The NK cells were treated or not with 15 μM IL-21 for 24 h. Shown is the average NK cell-mediated percent cytotoxicity for the NK cells of the six patients, with or without IL-21 treatment. (F) Effect of IL-21 on NK cell-mediated ADCC effector function. Isolated NK cells from three viremic, HAART-naïve, HIV-infected patients (P1–P3) were used in the ADCC assay, with or without treatment with IL-21 (15 μM) for 24 h. The NK cell-mediated ADCC was then measured against gp120/41-expressing CEM-gp120 cells in a standard $^{51}$Cr-release assay at an E:T ratio of 20:1 using medium containing diluted sera (5 μl) from HIV/AIDS patients (Patient serum) or from healthy subjects (Control serum). *, **, and ***, Statistically significant differences between indicated means ($P<0.05$, $P<0.01$, and $P<0.001$, respectively).

[22], we investigated the effect of the cytokine on CD16 expression in human NK cells. The cytokine did not affect the expression of this FcγR in any significant manner on human NK cells from healthy and HIV-infected individuals (data not shown).

We further sought to determine the effect of IL-21 treatment on NK cells with respect to their expression of perforin and IFN-γ: two molecules used as surrogate markers for NK cells' potential for cytotoxic and secretory functions, respectively. As shown in Figure 2C, the cytokine increased expression of perforin in NK92 as well as in $CD56^{bright}$ and $CD56^{dim}$ subsets of human primary NK cells to equivalent levels in in vitro cultures. The expression of this cytotoxic mediator was higher in $CD56^{dim}CD16^{+}$ cells as compared with $CD56^{bright}CD16^{+}$ NK cells in unstimulated cell cultures. The cytokine also increased expression of intracellular IFN-γ in these cultures. In this regard, $CD56^{bright}CD16^{+}$ expressed higher levels of the IFN intracellulary in unstimulated cell cultures, and more increase was seen in this parameter in these NK cells as a result of treatment with IL-21 (Fig. 2D). Even after stimulation with IL-21, the $CD56^{bright}$ subset expressed more intracellular IFN-γ as compared with the $CD56^{dim}$ subset. These data suggest that IL-21 has the ability to augment expression of these two effector molecules differentially in human NK cell subsets. These results are in agreement with those reported earlier [9, 16, 19].

One of the aims of this study was to determine if IL-21 could improve the ex vivo functions of NK cells from HIV-infected patients. Therefore, we isolated NK cells from viremic, HAART-naïve, HIV-infected patients and cultured them for 24 h with or without IL-21. After the incubation, we determined their natural cytotoxic activity as well as ADCC effector functions. As shown in Figure 2, E and F, IL-21 significantly enhanced natural cytotoxicity and ADCC effector function of these cells. In this respect, our results are in agreement with those of Strbo et al. [16], who have reported immune-enhancing effects of this cytokine on NK cells from HIV-infected individuals. However, we also show that the cytokine increases ADCC effector function of NK cells from HIV-infected patients.

### IL-21 exerts its effects on NK cells by activating STAT-3, ERK-1/2, p38, and Akt

To determine the signaling events associated with IL-21-augmented NK cell functions, we isolated human NK cells from freshly isolated PBMC and put them in culture with IL-21. At different time-points after addition of the cytokine, the cells were collected and washed, and their lysates were analyzed for the expression of STAT-3, ERK-1/ERK-2 (p44/42), p38, Akt, and their respective activation-associated, phosphorylated forms by Western blots using specific antibodies. As shown in **Figure 3A**, IL-21 increased levels of phosphorylated forms of STAT-3, p38, and the MAPKs as early as 5 min after addition of the cytokine. Increased levels of phosphorylated Akt were observed 1 h after the addition of the cytokine. The phosphorylated forms of these molecules were maintained at higher levels at each subsequently tested time-point. The cytokine treatment caused no visible increase in total levels of these proteins (Fig. 3A). The cytokine did not cause activation of stress-activated protein kinase/JNK and Vav (data not shown). IL-21 has been shown to phosphorylate and activate STAT-3 in NK cells [6, 16, 19, 23]. Our results confirm this and add further that the cytokine activates ERK-1, ERK-2, p38, and Akt but not JNK and Vav in these cells.

We also determine the effects of IL-21-mediated, enhanced activation of STAT-3, p44/42, p38, and Akt on different NK cell effector functions. For this purpose, we treated purified NK cells with IL-21 for 24 h, with or without the presence of pharmacological inhibitors specific for p42/44, p38, PI3K, and STAT-3 (U0126, SB230580, LY294002, and cucurbitacin, respectively), alone and in different combinations, and used them in these assays. As shown in Figure 3, B and C, the effect of STAT-3 inhibitor (cucurbitacin) was drastic on cytotoxicity and perforin expression. It completely inhibited an IL-21-mediated increase in cytotoxicity and perforin expression. In fact, it decreased these parameters even below the levels observed in the cytokine-untreated NK cells. Furthermore, it had no additive or synergistic effect with any other pathway inhibitor used here. On the other hand, the p44/42, p38, and PI3K-specific inhibitors behaved similar to each other. Each of them abrogated IL-21-mediated increases in cytotoxicity, perforin, and IFN-γ expression (Fig. 3, B–D). Of note, cucurbitacin behaved similarly to other inhibitors concerning its effects on the expression of IFN-γ in NK cells (Fig. 3D). The results from the STAT-3 inhibitor suggest that this molecule plays an important role in the constitutive and IL-21-enhanced functions of NK cells. To the best of our knowledge, this is first report linking STAT-3 activation with NK cell-mediated cytotoxicity.

### Effects of IL-21 on NK cell proliferation, viability, and expression of antiapoptotic proteins (Bcl-2 and Bcl-$X_L$)

Several γc-using cytokines, e.g., IL-2 and IL-15, are known to induce proliferation of NK cells [24]. Therefore, we sought to determine whether IL-21 also increases proliferation of these cells. For this purpose, we cultured freshly isolated human NK cells in the medium supplemented with equimolar concentrations of IL-2, IL-15, and IL-21. After 5 days of incubation, we measured cell proliferation via $^3$H-thymidine uptake. As shown in **Figure 4, A** and **B**, purified human NK cells from healthy donors as well as from HIV-infected patients, cultured in the presence of IL-2 and IL-15, resulted in a significant increase in their $^3$H-thymidine uptake. In contrast, the cells cultured with IL-21 had no increase in this parameter. However, IL-21 did not decrease $^3$H-thymidine uptake by NK cells when added to the cultures in combination with IL-2 or with IL-15. Rather, it enhanced the uptake when added to NK cells in combination with IL-2 and IL-15. These data suggest that IL-21 alone has minimal effect on the proliferation of NK cells but may act in synergy with IL-2 or IL-15 for enhancing NK cell proliferation. In this respect, our results are similar to those reported earlier [8].

Several studies have shown that absolute numbers as well as percentages of different lymphocytes are decreased over time in patients infected with HIV-1 [13] (reviewed in ref. [11]). NK cells and T cells from these individuals undergo enhanced

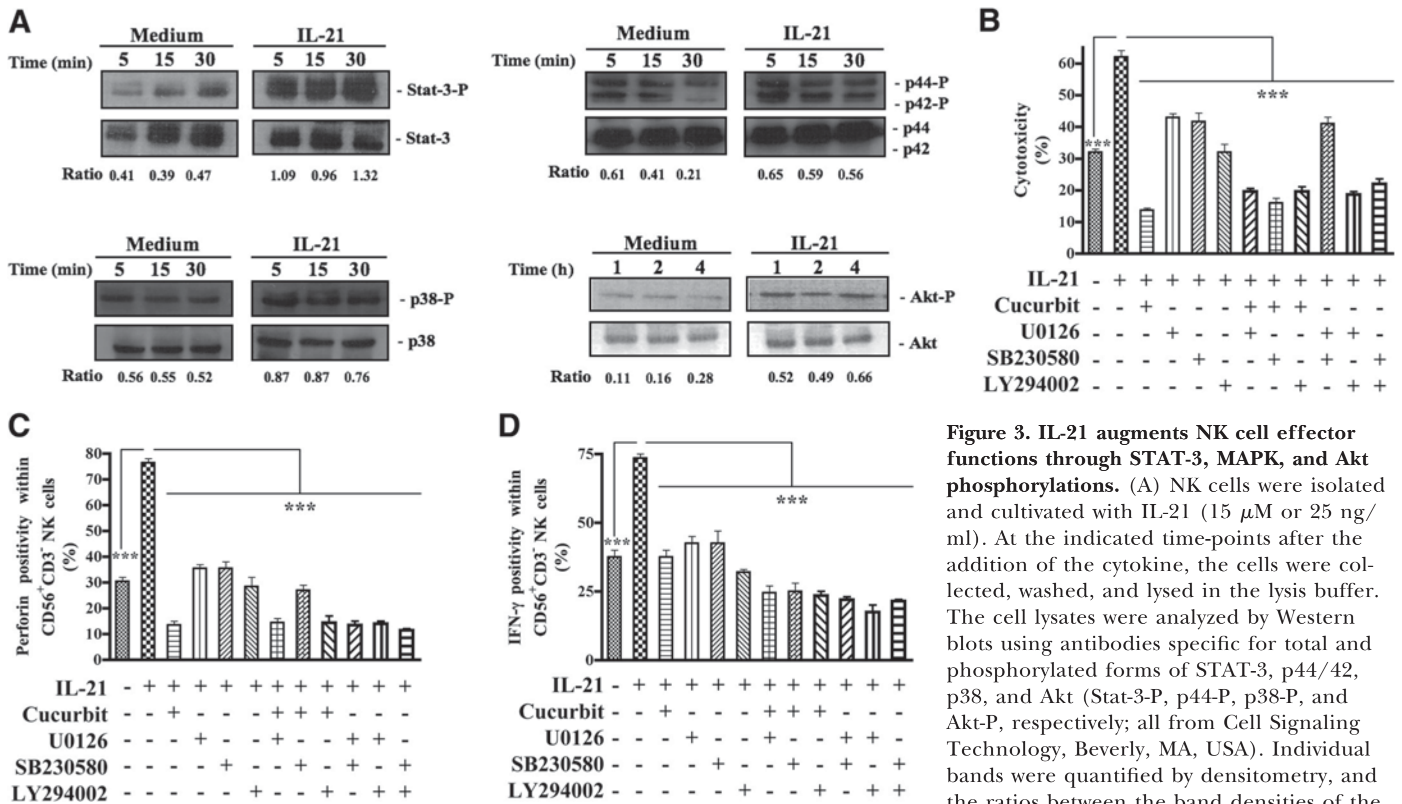


**Figure 3. IL-21 augments NK cell effector functions through STAT-3, MAPK, and Akt phosphorylations.** (A) NK cells were isolated and cultivated with IL-21 (15 μM or 25 ng/ml). At the indicated time-points after the addition of the cytokine, the cells were collected, washed, and lysed in the lysis buffer. The cell lysates were analyzed by Western blots using antibodies specific for total and phosphorylated forms of STAT-3, p44/42, p38, and Akt (Stat-3-P, p44-P, p38-P, and Akt-P, respectively; all from Cell Signaling Technology, Beverly, MA, USA). Individual bands were quantified by densitometry, and the ratios between the band densities of the phosphorylated and total proteins were determined and are shown at the lane bottoms. (B) Purified NK cells were treated with 15 μM IL-21 for 24 h, with or without the presence of specific inhibitors (10 μM each) for STAT-3 (cucurbitacin from Sigma-Aldrich, St. Louis, MO, USA), p44/42 (U0126 from Cell Signaling Technology), p38 (SB230580 from Cell Signaling Technology), and PI3K (LY294002 from Calbiochem, San Diego, CA, USA). The cytolytic potential of NK cells was then measured at a 20:1 E:T ratio against CEM targets. Average percent cytotoxicity ± SD from three replicates is shown in B. (C and D) Effects of IL-21 and the inhibitors on the expression of intracellular expression of perforin (C) and IFN-γ (D). All of these experiments were performed with NK cells from three different donors, and essentially similar results were obtained. ***, Statistically significant differences between indicated means ($P<0.001$).

spontaneous apoptosis when cultured ex vivo [25–27]. The use of HAART is effective in these patients in reducing viral load but is slow in restoring NK and $CD4^+$ T cell compartments to their physiological levels. The restoration of their viability in HIV-infected AIDS patients is a real challenge. This has led researchers to consider using immune-enhancing cytokines as a therapeutic agent. In this regard, members of the γc-using cytokine family have been shown to play a crucial role in NK cell homeostasis by enhancing their proliferation and survival [8, 26–28]. As IL-21 belongs to this family of cytokines, we sought to determine whether it could also promote NK cell survival and hence, contribute to NK cell homeostasis. For this purpose, we purified NK cells from freshly isolated human PBMC and cultured them in the presence of cytokines under suboptimal conditions (RPMI 1640 with 1% FCS). We added equimolar concentrations of IL-2, IL-15, or IL-21 to these cultures separately as well as in combination with IL-15 or IL-2. We measured the percentages of NK cells undergoing apoptosis by flow cytometry 72 h later. As shown in Figure 4C, IL-21 treatment reduced the percentages of NK cells undergoing spontaneous apoptosis from healthy donors in these cell cultures. In this respect, IL-21 was consistently more effective than IL-2. The cytokine was at least as effective as IL-15 in preventing spontaneous apoptosis in ex vivo-incubated human NK cells. No additive or synergistic prosurvival effects were observed when IL-21 was added to these cultures in combination with IL-2 or with IL-15.

We next investigated if this prosurvival effect of IL-21 could be associated with a modulation in the expression of one or more apoptosis-regulating proteins belonging to the Bcl-2 family. To determine this, we performed Western blots for Bcl-2 and $Bcl\text{-}X_L$ on IL-21-treated and untreated, purified NK cells, and the results from a typical experiment are shown in Figure 4D. It is quite evident from this experiment that IL-21 increases and maintains higher levels of expression of $Bcl\text{-}X_L$ and Bcl-2 proteins in human NK cells. Similar results were obtained when these experiments were repeated with NK cells obtained from two other donors (data no shown). We show here for the first time that IL-21 enhances the expression of these two antiapoptotic proteins in vitro in human NK cells. These prosurvival effects of IL-21 for human NK cells are similar to the ones that have been reported earlier for $CD4^+$ and $CD8^+$ T cells [29, 30]. In separate experiments, we investigated the effects of IL-21 on spontaneous mortality in NK cells

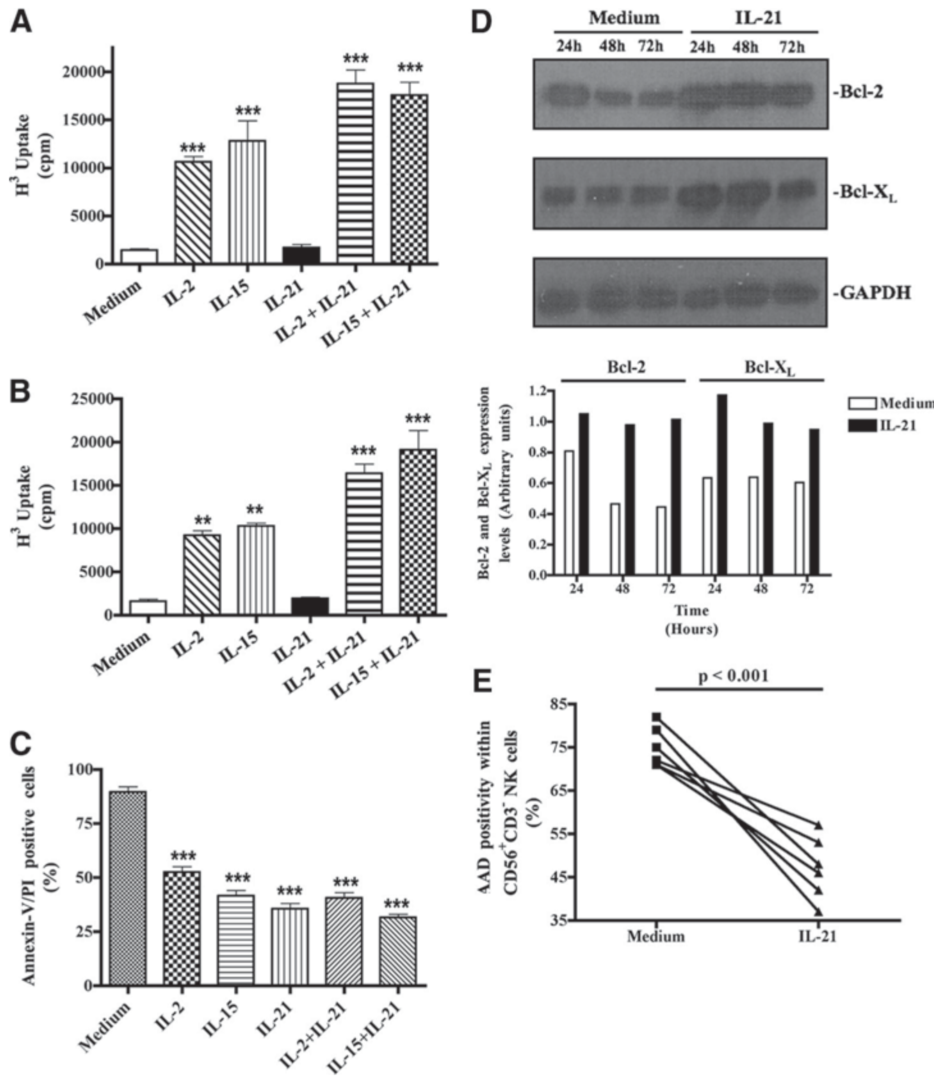


**Figure 4. IL-21 does not enhance proliferation but acts as a prosurvival factor for NK cells and increases expression of Bcl-2 and Bcl-$X_L$.** (A and B) Purified NK cells from healthy donors (A) and viremic, HIV-infected patients (B) were cultured in the medium containing 15 $\mu$M IL-21 and/or other cytokines as indicated. Five days later, 50 $\mu$l medium containing 1 $\mu$Ci $^3$H-thymidine (Amersham Biosciences) was added to the culture wells for 4 h. The cells were then harvested, and the incorporation was measured by a liquid scintillation counter. Similar results were obtained with three different healthy donors and three HIV-infected patients. (C) Purified NK cells from healthy donors were cultured in suboptimal medium (RPMI 1640 with 1% FCS), with or without equimolar concentrations (15 $\mu$M) of the indicated cytokines. After 72 h, the cells were analyzed for the expression of apoptosis by flow cytometry after staining with FITC-Annexin V and propidium iodide (PI; from BD Biosciences). (D) The cell lysates from IL-21-treated and untreated cells were analyzed by Western blotting using mAb specific for Bcl-2 (from Calbiochem), Bcl-$X_L$ (from Cell Signaling Technology), and GAPDH (from Ambion, Austin, TX, USA). Individual bands were quantified by densitometry, and the expression of these proteins relative to that of GAPDH was plotted for each time-point (lower panel). Similar results were obtained with three different healthy donors. (E) The PBMC from six viremic, HAART-naïve, HIV-infected patients were cultured in RPMI 1640 containing 1% FCS, with or without 15 $\mu$M IL-21. After 72 h, the cells were harvested and stained using FITC-conjugated anti-CD56 and PE-conjugated anti-CD3 antibodies (all from eBioscience) and 7-AAD (from BD Biosciences). The figure shows the percentage of 7-AAD-positive (dead) cells for each patient, with or without treatment with the cytokine for the NK cell subset that has been shown to decrease in HIV-infected patients [13]. ** and ***, Statistically significant differences between means ($P<0.01$ and $P<0.001$, respectively).

isolated from six HIV-infected, HAART-naïve, viremic patients. As shown in Figure 4E, the cytokine decreased spontaneous mortality of NK cells from these patients, as determined by 7-AAD staining. Taken together, these results show that IL-21 is able to improve the survival of NK cells significantly from healthy donors as well as from HIV-infected patients in ex vivo experiments.

### Il-21 has little effects on HIV-1 replication

Despite their positive effect on the antiviral immunity, one of the disadvantages of the use of biological response modifiers is their ability to increase HIV-1 replication. In this respect, IL-15 was shown to have relatively less enhancing effects on HIV-1 replication as compared with IL-2 and IL-12 [31, 32]. IL-7 was also reported to have this undesirable effect in HIV-infected individuals [33]. As a result of its less cell-proliferative effects as compared with IL-2 or IL-15, we sought to determine if IL-21 also exerted less stimulatory effects on HIV-1 replication in human cells. To this end, we infected T cell blasts obtained from PHA and IL-2-activated PBMC of healthy donors in vitro with a T-tropic HIV-1 strain (NL4.3). After washing to remove the residual virus, the infected cells were cultured in the presence of cytokines. After 24, 48, and 72 h, the culture supernatants were collected and analyzed for their p24 contents. As shown in **Figure 5, A** and **B,** IL-21 had the least-stimulatory effects on HIV-1 replication when p24 was measured in the culture supernatants at all of the three tested time-points. When added alone, IL-2 and IL-15 increased HIV-1 replication two- to threefold more than IL-21. Interestingly, IL-21 did not act synergistically or additively with IL-2 or with IL-15 to stimulate the viral replication (Fig. 5, A and B). Furthermore, increasing the dose of IL-21 in these experiments did not cause a significant increase in HIV-1 replication,

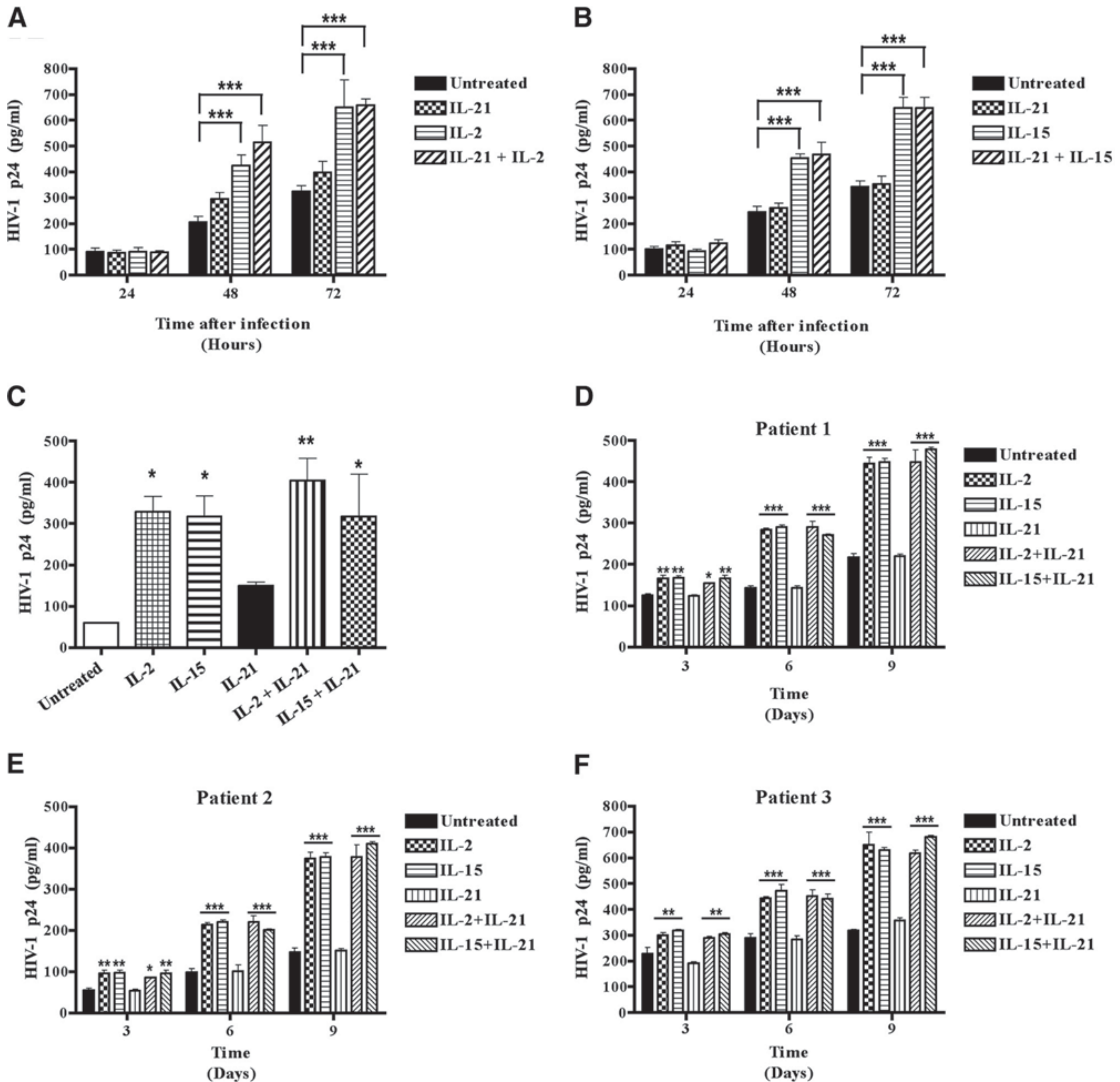


**Figure 5. IL-21 has less of a HIV-1 replication-enhancing effect as compared with IL-2 and IL-15.** PBMC from HIV-seronegative, healthy donors were activated with PHA (10 $\mu$g/ml) and IL-2 (100 U/ml) for 5 days. After 5 days, the activated PBMC were infected in vitro with HIV-1 (NL4.3) for 2 h at 37°C, and cells were then washed extensively to remove residual virus. The cells were then cultivated with equimolar concentrations of different cytokines (15 $\mu$M) as indicated for 24, 48, and 72 h. For each time-point, culture supernatants were collected and centrifuged to remove cell debris, and their HIV-1 p24 contents were measured by ELISA, obtained from the AIDS Vaccine Program (National Cancer Institute, Frederick, MD, USA). (A) The effects of IL-21 alone and in combination with IL-2; (B) the effects of IL-21 alone and in combination with IL-15. Similar results were obtained with three different donors. (C) Activated PBMC from normal donors were treated with equimolar concentrations (15 $\mu$M) of the indicated cytokines for 24 h. The cells were then infected with NL4.3, washed, and cultured for 48 h in the presence of cytokines, as described in the above section. After 48 h, the p24 was measured in the culture supernatants by ELISA. Note less enhancement of HIV-1 replication by IL-21 as compared with the other two cytokines. Similar results were obtained with three different donors. (D–F) The PBMC from three HAART-naïve, viremic patients (with 367,168, 742,290, and 211,120 copies of viral RNA/ml plasma in Patients 1, 2, and 3, respectively) were activated with PHA (10 $\mu$g/ml) and IL-2 (100 U/ml). After 10 days, the cells were washed, and the cytokines were added to the cell cultures as indicated at equimolar (15 $\mu$M) concentrations. After 3, 6, and 9 days, the p24 was measured in the culture supernatants by ELISA. *, **, and ***, Statistically significant differences between indicated means concentrations of p24 in the culture supernatants ($P<0.05$, $P<0.01$, and $P<0.001$, respectively).

whereas increasing the dose of IL-2 or IL-15 resulted in enhanced viral replication (data not shown). The cytokine also had little enhancing effects on monocyte-tropic (R5) HIV-1 strains in human macrophages (data not shown). We further determined the effects of the treatments of PBMC with these cytokines prior to infecting them with HIV-1. As shown in Figure 5C, the pretreatment of the cells by IL-21 before the infection also had less-enhancing effects on HIV-1 replication as compared with the pretreatments with IL-2 or IL-15.

We next determined if IL-21 increased the production of endogenous HIV-1 in PBMC from viremic, HIV-infected patients. Therefore, we isolated PBMC from three different HAART-naïve, viremic, HIV-infected patients and activated them with PHA and IL-2 for 10 days. After this activation, the cells were washed and cultivated with equimolar concentrations of IL-2, IL-15, or IL-21 for 3, 6, and 9 days. As shown in Figure 5, D–F, IL-21 had little effect on HIV-1 replication at all of the tested time-points, whereas IL-2 and IL-15 increased HIV-1 replication progressively in the PBMC of all the donors at the three tested time-points. Furthermore, IL-21 had no additive or synergistic effects on HIV-1 replication when it was added to the cell cultures from the patients in combination with IL-2 or with IL-15. Taken together, these data show that IL-21 induces little HIV-1 replication alone as well in combination with other immune-enhancing cytokines such as IL-2 and IL-15. To the best of our knowledge, this is the first report describing minimal effects of this cytokine on HIV-1 replication.

## IL-21 increases NK cell-mediated suppression of HIV-1 replication in CD4$^+$ T cells

Studies have shown that NK cells can suppress HIV-1 replication when they are cocultured with HIV-infected CD4$^+$ T cells [34, 35]. Therefore, we further sought to determine the effect of IL-21 on NK cell-mediated suppression of HIV-1 replication in the coculture experiments. For this purpose, we isolated CD4$^+$ T cells from PHA and IL-2-activated human PBMC, infected them with HIV-1, and coincubated them with purified, autologous NK cells at a 1:1 ratios. We added IL-21 to these cocultures and incubated them at 37°C in a humidified atmosphere containing 5% $CO_2$. We obtained culture supernatants from these cocultures at different time-points, cleared them from cell debris, and analyzed them for p24 contents. As shown in **Figure 6A**, IL-21 did not affect viral replication in HIV-infected CD4$^+$ T cells but significantly enhanced NK cell-mediated suppression of HIV-1 replication at Days 5, 7, and 9 after start of the cocultures ($P<0.001$).

To investigate the underlying mechanism of the NK cell-mediated suppression of HIV-1 replication in the coculture experiments, we repeated the experiments as described above and added neutralizing antibodies against IFN-γ, perforin, LFA-1, or control antibodies. After 5 days of incubation, we collected cell culture supernatants and analyzed them for p24 assay. As shown in Figure 6B, the antibodies against LFA-1 and to a lesser extent, against perforin, but not against IFN-γ, abrogated the suppressive effect of NK cells. Essentially similar results were obtained when the cocultures were maintained for 9 days and examined for their p24 contents in the supernatants (data not shown). It is noteworthy that IL-21 did not render human CD4$^+$ T cells more susceptible (with or without infection with HIV-1) to NK cell-mediated cytolysis (data not shown).

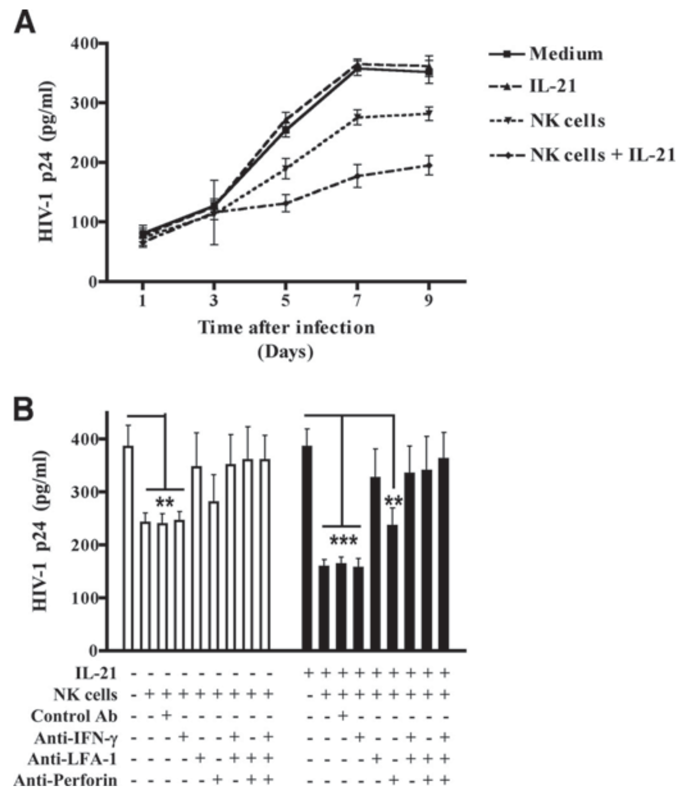


**Figure 6. IL-21 increases NK cell-mediated suppression of HIV-1 replication in vitro.** (A) PBMC from four HIV-seronegative, healthy donors were activated with PHA (10 μg/ml; from Sigma-Aldrich) and IL-2 (100 U/ml). After 5 days, activated CD4$^+$ T cells were isolated and infected with HIV-1 (NL4.3) for 2 h at 37°C. Cells were then washed extensively to remove the residual virus. Equal numbers of purified, autologous NK cells (ratio of 1:1) were cocultured with the HIV-infected CD4$^+$ T cells, with or without (15 μM) IL-21. On Days 1, 3, 5, 7, and 9 after infection, the culture supernatants from the cocultures were assayed for their p24 contents. The figure shows averages ± SD p24 contents from four different donors at each time-point. (B) Molecular mechanisms underlying the IL-21-enhanced NK cell-mediated suppression of HIV-1 replication in CD4$^+$ T cells. Autologous NK cells and HIV-infected CD4$^+$ T cells were cocultured with and without IL-21 as described in A. Neutralizing antibodies against LFA-1, IFN-γ, or isotype-matched mouse IgG (Control Ab) were added at 1 μg/ml concentrations as indicated. All of these antibodies were from BD Biosciences. Seven days later, HIV-1 replication in the cocultures was measured by p24 ELISA. The figure shows averages ± SD p24 contents from four different donors. Note abrogation of the NK cell-mediated suppression of HIV-1 replication by anti-LFA-1 but not by anti-IFN-γ antibodies. Essentially, similar results were obtained for the four different donors when cocultures were prolonged for 9 days (data not shown). ** and ***, Statistically significant differences between means ($P<0.01$ and $P<0.001$, respectively).

As LFA-1 is needed for conjugate formation between NK and target cells, these data suggest that physical contact and hence, NK cell-mediated cytotoxicity are essential for viral suppression in these experiments. It is noteworthy that the interaction of LFA-1 with ICAM-3 on target cells is essential for conjugate formation between NK and target cells [36, 37]. This interaction also acts as a trigger for NK cell degranulation [38]. The fact that anti-LFA-1, but not antiperforin, antibodies block HIV-1 replication completely in this coculture system suggests that the former antibodies may also be inhibiting cell–cell spread of the virus. It is noteworthy that LFA-1 plays a role in this mode of HIV infection [39]. Our in vitro experiments show that IL-21 has no effect on the expression of LFA-1 on NK cells (data not shown). However, it still remains to be investigated whether it causes any conformational changes and increases affinity of this adhesion molecule for its ligand. The results from the coculture system suggest that IL-21-treated NK cells can kill HIV-infected, autologous $CD4^+$ T cells efficiently. Thus, IL-21 may be used to enhance NK cell functions and control HIV-1 replication in HIV-infected individuals.

Collectively, our results suggest that IL-21 may represent a valuable, therapeutic tool for augmenting survival and antiviral effects of NK cells in HIV-infected patients. It is noteworthy that similar immune-enhancing effects of IL-21 have also been described for $CD8^+$ T cells from HIV-infected persons [30]. It may be worth mentioning that the cytokine is well-tolerated by humans in therapeutic doses as compared with similar other cytokines and is currently being tested in clinical trials for immunotherapy against melanoma and renal cell carcinoma [40]. Given that IL-21 concentrations decrease in the circulation of HIV-infected individuals, immunotherapy with this cytokine may be more relevant and appropriate in the AIDS patients.

## AUTHORSHIP

A. I. and S. S. performed the experiments. A. I., M-R. B., S. S., and A. A. designed experiments, collected data, and wrote the manuscript. M-R. B., C. T., E. T., and J-P. R. collected and provided PBMC from HIV/AIDS patients and reviewed the manuscript. A. A. supervised the project and compiled the final version of the manuscript.

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### DISCLOSURE

The authors declare no competing financial interests.

### REFERENCES

1. Spolski, R., Leonard, W. J. (2008) Interleukin-21: basic biology and implications for cancer and autoimmunity. *Annu. Rev. Immunol.* **26,** 57–79.
2. Parrish-Novak, J., Dillon, S. R., Nelson, A., Hammond, A., Sprecher, C., Gross, J. A., Johnston, J., Madden, K., Xu, W., West, J., et al. (2000) Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. *Nature* **408,** 57–63.
3. Onoda, T., Rahman, M., Nara, H., Araki, A., Makabe, K., Tsumoto, K., Kumagai, I., Kudo, T., Ishii, N., Tanaka, N., Sugamura, K., Hayasaka, K., Asao, H. (2007) Human CD4+ central and effector memory T cells produce IL-21: effect on cytokine-driven proliferation of CD4+ T cell subsets. *Int. Immunol.* **19,** 1191–1199.
4. Yi, J. S., Du, M., Zajac, A. J. (2009) A vital role for interleukin-21 in the control of a chronic viral infection. *Science* **324,** 1572–1576.
5. Nutt, S. L., Brady, J., Hayakawa, Y., Smyth, M. (2004) Interleukin 21: a key player in lymphocyte maturation. *Crit. Rev. Immunol.* **24,** 239–250.
6. Burgess, S. J., Marusina, A. I., Pathmanathan, I., Borrego, F., Coligan, J. E. (2006) IL-21 down-regulates NKG2D/DAP10 expression on human NK and CD8+ T cells. *J. Immunol.* **176,** 1490–1497.
7. Eriksen, K. W., Søndergaard, H., Woetmann, A., Krejsgaard, T., Skak, K., Geisler, C., Wasik, M., Odum, N. (2009) The combination of IL-21 and IFN-$\alpha$ boosts STAT3 activation, cytotoxicity and experimental tumor therapy. *Mol. Immunol.* **46,** 812–820.
8. Skak, K., Frederiksen, K., Lundsgaard, D. (2008) Interleukin-21 activates human natural killer cells and modulates their surface receptor expression. *Immunology* **123,** 575–583.
9. Strengell, M., Matikainen, S., Siren, J., Lehtonen, A., Foster, D., Julkunen, I., Sareneva, T. (2003) IL-21 in synergy with IL-15 or IL-18 enhances IFN-{$\gamma$} production in human NK and T cells. *J. Immunol.* **170,** 5464–5469.
10. Wang, G., Tschoi, M., Spolski, R., Lou, Y., Ozaki, K., Feng, C., Kim, G., Leonard, W. J., Hwu, P. (2003) In vivo antitumor activity of interleukin 21 mediated by natural killer cells. *Cancer Res.* **63,** 9016–9022.
11. Iannello, A., Debbeche, O., Samarani, S., Ahmad, A. (2008) Antiviral NK cell responses in HIV infection: II. viral strategies for evasion and lessons for immunotherapy and vaccination. *J. Leukoc. Biol.* **84,** 27–49.
12. Scott-Algara, D., Truong, L. X., Versmisse, P., David, A., Luong, T. T., Nguyen, N. V., Theodorou, I., Barre-Sinoussi, F., Pancino, G. (2003) Cutting edge: increased NK cell activity in HIV-1-exposed but uninfected Vietnamese intravascular drug users. *J. Immunol.* **171,** 5663–5667.
13. Mavilio, D., Lombardo, G., Benjamin, J., Kim, D., Follman, D., Marcenaro, E., O'Shea, M. A., Kinter, A., Kovacs, C., Moretta, A., Fauci, A. S. (2005) Characterization of CD56–/CD16+ natural killer (NK) cells: a highly dysfunctional NK subset expanded in HIV-infected viremic individuals. *Proc. Natl. Acad. Sci. USA* **102,** 2886–2891.
14. Iannello, A., Tremblay, C., Routy, J., Boulassel, M., Toma, E., Ahmad, A. (2008) Decreased levels of circulating IL-21 in HIV-infected AIDS patients: correlation with CD4+ T cell counts. *Viral Immunol.* **21,** 385–388.
15. Iannello, A., Boulassel, M., Samarani, S., Debbeche, O., Tremblay, C., Toma, E., Routy, J., Ahmad, A. (2010) Dynamics and consequences of IL-21 production in HIV-infected individuals: a longitudinal and cross-sectional study. *J. Immunol.* **184,** 114–126.
16. Strbo, N., de Armas, L., Liu, H., Kolber, M., Lichtenheld, M., Pahwa, S. (2008) IL-21 augments natural killer effector functions in chronically HIV-infected individuals. *AIDS* **22,** 1551–1560.
17. Ahmad, A., Morisset, R., Thomas, R., Menezes, J. (1994) Evidence for a defect of antibody-dependent cellular cytotoxic (ADCC) effector function and anti-HIV gp120/41-specific ADCC-mediating antibody titres in HIV-infected individuals. *J. Acquir. Immune Defic. Syndr.* **7,** 428–437.
18. Zhang, Y., Stastny, P. (2006) MICA antigens stimulate T cell proliferation and cell-mediated cytotoxicity. *Hum. Immunol.* **67,** 215–222.
19. Wendt, K., Wilk, E., Buyny, S., Schmidt, R.E., Jacobs, R. (2007) Interleukin-21 differentially affects human natural killer cell subsets. *Immunology* **122,** 486–495.
20. Brady, J., Hayakawa, Y., Smyth, M., Nutt, S. (2004) IL-21 induces the functional maturation of murine NK cells. *J. Immunol.* **172,** 2048–2058.
21. Roda, J. M., Parihar, R., Lehman, A., Mani, A., Tridandapani, S., Carson III, W. E. (2006) Interleukin-21 enhances NK cell activation in response to antibody-coated targets. *J. Immunol.* **177,** 120–129.
22. Iannello, A., Ahmad, A. (2005) Role of antibody-dependent cell-mediated cytotoxicity in the efficacy of therapeutic anti-cancer monoclonal antibodies. *Cancer Metastasis Rev.* **24,** 487–499.
23. Strengell, M., Sareneva, T., Foster, D., Julkunen, I., Matikainen, S. (2002) IL-21 up-regulates the expression of genes associated with innate immunity and Th1 response. *J. Immunol.* **169,** 3600–3605.
24. Grund, E. M., Spyropoulos, D., Watson, D., Muise-Helmericks, R. (2005) Interleukins 2 and 15 regulate Ets1 expression via ERK1/2 and MNK1 in human natural killer cells. *J. Biol. Chem.* **280,** 4772–4778.
25. Naora, H., Gougeon, M. (1999) Interleukin-15 is a potent survival factor in the prevention of spontaneous but not CD95-induced apoptosis in CD4 and CD8 T lymphocytes of HIV-infected individuals. Correlation with its ability to increase BCL-2 expression. *Cell Death Differ.* **6,** 1002–1011.

26. Naora, H., Gougeon, M. (1999) Enhanced survival and potent expansion of the natural killer cell population of HIV-infected individuals by exogenous interleukin-15. *Immunol. Lett.* **68,** 359–367.
27. Vassena, L., Proschan, M., Fauci, A., Lusso, P. (2007) Interleukin 7 reduces the levels of spontaneous apoptosis in CD4+ and CD8+ T cells from HIV-1-infected individuals. *Proc. Natl. Acad. Sci. USA* **104,** 2355–2360.
28. Pahwa, S. (2007) Role of common γ chain utilizing cytokines for immune reconstitution in HIV infection. *Immunol. Res.* **38,** 373–386.
29. Ostiguy, V., Allard, E.-L., Marquis, M., Leignadier, J., Labrecque, N. (2007) IL-21 promotes T lymphocyte survival by activating the phosphatidylinositol-3 kinase signaling cascade. *J. Leukoc. Biol.* **82,** 645–656.
30. White, L., Krishnan, S., Strbo, N., Liu, H., Kolber, M. A., Lichtenheld, M. G., Pahwa, R. N., Pahwa, S. (2007) Differential effects of IL-21 and IL-15 on perforin expression, lysosomal degranulation, and proliferation in CD8 T cells of patients with human immunodeficiency virus-1 (HIV). *Blood* **109,** 3873–3880.
31. Bayard-McNeeley, M., Doo, H., He, S., Hafner, A., Johnson Jr., W. D., Ho, J. L. (1996) Differential effects of interleukin-12, interleukin-15, and interleukin- 2 on human immunodeficiency virus type 1 replication in vitro. *Clin. Diagn. Lab. Immunol.* **3,** 547–553.
32. Patki, A. H., Quiñones-Mateu, M., Dorazio, D., Yen-Lieberman, B., Boom, W., Thomas, E., Lederman, M. (1996) Activation of antigen-induced lymphocyte proliferation by interleukin-15 without the mitogenic effect of interleukin-2 that may induce human immunodeficiency virus-1 expression. *J. Clin. Invest.* **98,** 616–621.
33. Smithgall, M. D., Wong, J. G., Critchett, K. E., Haffar, O. K. (1996) IL-7 up-regulates HIV-1 replication in naturally infected peripheral blood mononuclear cells. *J. Immunol.* **156,** 2324–2330.
34. Bonaparte, M. I., Barker, E. (2004) Killing of human immunodeficiency virus-infected primary T-cell blasts by autologous natural killer cells is dependent on the ability of the virus to alter the expression of major histocompatibility complex class I molecules. *Blood* **104,** 2087–2094.
35. Fogli, M., Mavilio, D., Brunetta, E., Varchetta, S., Ata, K., Roby, G., Kovacs, C., Follmann, D., Pende, D., Ward, J., Barker, E., Marcenaro, E., Moretta, A., Fauci, A. (2008) Lysis of endogenously infected CD4+ T cell blasts by rIL-2 activated autologous natural killer cells from HIV-infected viremic individuals. *PLoS Pathog.* **4,** e1000101.
36. Barber, D. F., Faure, M., Long, E. O. (2004) LFA-1 contributes an early signal for NK cell cytotoxicity. *J. Immunol.* **173,** 3653–3659.
37. Liu, D., Bryceson, Y. T., Meckel, T., Vasiliver-Shamis, G., Dustin, M. L., Long, E. O. (2009) Integrin-dependent organization and bidirectional vesicular traffic at cytotoxic immune synapses. *Immunity* **31,** 99–109.
38. Perez, O. D., Mitchell, D., Jager, G. C., Nolan, G. P. (2004) LFA-1 signaling through p44/42 is coupled to perforin degranulation in CD56+CD8+ natural killer cells. *Blood* **104,** 1083–1093.
39. Hioe, C. E., Chien Jr., P. C., Lu, C., Springer, T. A., Wang, X-H., Bandres, J., Tuen, M. (2001) LFA-1 expression on target cells promotes human immunodeficiency virus type 1 infection and transmission. *J. Virol.* **75,** 1077–1082.
40. Davis, I. D., Brady, B., Kefford, R., Millward, M., Cebon, J., Skrumsager, B., Mouritzen, U., Hansen, L., Skak, K., Lundsgaard, D., Frederiksen, K., Kristjansen, P., McArthur, G. (2009) Clinical and biological efficacy of recombinant human interleukin-21 in patients with stage IV malignant melanoma without prior treatment: a phase IIa trial. *Clin. Cancer Res.* **15,** 2123–2129.

KEY WORDS:
LFA-1 · MAPK · perforin · STAT-3

# 13- Discussion

## 13-1 Discussion sur les études concernant l'IL-18

Lors de notre étude, nous avons démontré qu'il existe une corrélation négative entre le nombre des cellules NK présentes en périphérie et les niveaux d'IL-18 circulantes chez les patients infectés par le VIH-1. Cette corrélation inverse a été observée au niveau de tous les sous-types majeurs de cellules NK étudiés ($CD3^-CD16^+$, $CD3^-CD56^+$, $CD3^-CD16^+CD56^+$) chez ces patients. Nos données ont donc suggéré que l'IL-18 est impliquée dans la perte du nombre de cellules NK chez les personnes infectées par le VIH-1, et que l'ensemble des différents sous-types de cellules NK sont affectés par cette cytokine. Notre étude a de plus permis de comprendre les mécanismes impliqués concernant la perte de ces cellules NK (Figure 13). Nous avons démontré que l'IL-18 induit une mort fratricide de ces cellules en induisant l'expression de la protéine FasL et en augmentant la sécrétion de TNF-$\alpha$. Des résultats similaires ont été obtenus lors de nos expériences sur des cellules NK primaires ainsi que sur la lignée cellulaire NK92. Le traitement de ces cellules avec de l'IL-18 n'a cependant pas augmenté l'expression de Fas et du récepteur au TNF-$\alpha$. Malgré une augmentation de l'expression en surface de la protéine FasL, nous n'avons pas observé d'augmentation dans la sécrétion de la forme soluble de la protéine FasL dans les surnageants de cultures des cellules traitées avec l'IL-18. De plus, ces surnageants n'ont pas été capable d'induire de mort cellulaire lorsqu'on les a ajoutés à des cellules NK fraîchement isolées ou aux cellules NK92 (ces données n'ont pas été publiées). Nos études ont aussi permis de mettre en évidence le fait que l'IL-18 augmente la susceptibilité des cellules NK à un stimuli apoptotique en réduisant l'expression de la protéine Bcl-$X_L$ au niveau des cellules NK primaires et des cellules NK92. Aucune réduction dans l'expression de la protéine Bcl-2 a été observée. Cette étude a démontré pour la première fois l'implication de l'IL-18 dans la sous-régulation d'une protéine anti-apoptotique au niveau des cellules NK. Il serait d'ailleurs intéréssant de déterminer si cette cytokine induit des réductions similaires concernant cette famille de protéines anti-apoptotiques au niveau des cellules T. Nous avons démontré que l'IL-18 causait la mort des cellules NK92 et des cellules

NK primaires d'une façon dose-dépendante. Même lors de l'utilisation de concentrations faibles d'IL-18 (100 pg/ml), cette cytokine a causé une mort cellulaire significative. Cependant, lorsque nous avons réalisé le même genre d'expériences en utilisant des sérums contenant de l'IL-18, seulement les sérums contenant 500 pg/ml et plus d'IL-18 circulantes ont permis de causer une mort cellulaire. Les sérums contenant des niveaux faibles d'IL-18 n'ont pas permis une mort fratricide des cellules NK en culture. Il est possible que la présence d'autres facteurs solubles sériques comme l'IL-18BP, des cytokines, ou des facteurs de croissances dans ces sérums aient pu affecter l'effet de l'IL-18. Cet effet mortel de l'IL-18 sur les cellules NK pourrait en fait représenter un mécanisme de rétro-contrôle négatif permettant de clore la réponse immunitaire inflammatoire. Il a été démontré dans le passé que la combinaison d'IL-12 et d'IL-15, ainsi que d'IL-2 et d'IL-12, pouvait causer la mort des cellules NK dépendante du TNF-$\alpha$ [381]. Les auteurs de cette étude n'ont cependant pas impliqué la voie Fas/FasL dans cette mort induite par ces cytokines. Concernant notre étude, nous avons aussi investigué si la voie des TRAIL pouvait être impliquée dans la mort induite par l'IL-18. Cependant, cette voie apoptotique n'a pas semblé être impliquée dans la mort induite par l'IL-18. Il est important de noter que l'IL-18, et non l'IL-2 ou l'IL-15, a été démontrée comme une cytokine ayant la capacité d'augmenter la mort cellulaire de cellules NK murines et de cellules T de type Th1 en utilisant la voie Fas/FasL [382-384]. Cependant, l'expression de FasL n'a pas été mesurée dans leurs expériences. Lors de notre étude, nous avons démontré pour la première fois que l'IL-18 induit l'expression de FasL à la surface de cellules NK primaires ainsi que la lignée NK92. Ces cellules expriment faiblement FasL de façon constitutive. Nous avons démontré que l'IL-18 active le promoteur du gène codant pour FasL lors d'expériences utilisant un gène rapporteur correspondant à cette protéine. Il est donc concevable que l'expression de FasL induite par l'IL-18 devrait résulter sur une augmentation de l'activité cytotoxique des cellules NK. Cependant, ces cellules $FasL^+$ pourraient aussi causer la destruction de tissus et de différentes cellules qui expriment le récepteur Fas de façon constitutive. En effet, l'IL-18 a été impliquée dans des dommages médiés par FasL et le TNF-$\alpha$ après induction par une endotoxine au niveau du foie chez la souris [385, 386]. Une étude a démontré que l'IL-18 empêchait l'apoptose des cellules NK lors de la lyse des cellules cibles [387]. Les auteurs de cette

étude ont attribué cet effet protecteur de l'IL-18 à une augmentation de l'expression des protéines TRAF-1 (*TNF Receptor Associated Factor-1*) et c-IAP (*cellular-Inhibitor of Apoptosis*). Une autre étude a montré que l'IL-18 induisait l'expression de CD83, CCR7 et CD25 à la surface des cellules NK et augmentait leur capacité de migration vers les noeuds lymphatiques afin d'interagir avec d'autres immunocytes [388]. L'effet de l'IL-18 sur ces fonctions *helper* concernant les cellules NK reste uniquement démontré lors de cette étude. Lors de notre étude, nous avons examiné la mort des cellules NK en réalisant des microcultures lors desquelles les cellules ont pu interagir entre elles après un traitement avec l'IL-18. Notre étude ne contredit donc en rien les observations réalisées par ces équipes.

Nos résultats concernant l'augmentation de la concentration sérique de l'IL-18 chez les patients infectés par le VIH-1 confirment les résultats d'études antérieures réalisées par mon laboratoire d'accueil ainsi que par d'autres équipes [389-392]. Nos résultats démontrent que la traitement antirétroviral (HAART) ne permet pas une normalisation de ces niveaux, et ce même chez les patients pour qui le traitement est efficace avec une charge virale indétectable. Des études récentes ont démontré que certaines drogues antivirales utilisées lors du traitement, comme les inhibiteurs de protéase, induisent la sécrétion de protéines inflammatoires comme l'IL-1$\beta$, le TNF-$\alpha$ et l'IL-6 [393]. Sachant que l'IL-1$\beta$ et l'IL-18 appartiennent à la même famille et partagent des mécanismes similaires de production et de maturation, il serait donc possible que ces drogues augmentent aussi la production d'IL-18 lors du traitement, notamment au niveau des macrophages. Nous avons démontré pour la première fois que, de façon concomitante à une augmentation des niveaux sériques d'IL-18, une réduction des niveaux sériques d'IL-18BP a lieu chez ces mêmes patients infectés par le VIH-1 (Figure 13). La conséquence directe de la baisse de l'antagoniste circulant naturel de l'IL-18 et du déséquilibre entre ces deux facteurs chez les patients correspond à une augmentation des niveaux sériques de l'IL-18 libre, non liée à l'IL-18BP, et donc biologiquement active. Ce déséquilibre résulte sur une augmentation de l'activité biologique de l'IL-18 circulante chez les patients infectés. Une autre étude a déjà montré que l'activité biologique de cette cytokine est augmentée dans les sérums d'individus

infectés par le VIH-1 [389]. Lors de notre étude, nous avons démontré que l'infection des macrophages par le VIH-1 pouvait contribuer au déséquilibre dans la production d'IL-18 et d'IL-18BP chez les patients infectés. L'infection de ces cellules, présentes dans différents tissus du corps humain, a d'ailleurs lieu *in vivo* [394]. Il est d'ailleurs établi que les macrophages sont parmis les premières cellules à être infectées au niveau des muqueuses, ce qui représente d'ailleurs la voie prédominante d'infection chez l'homme. Ces cellules représentent aussi un réservoir d'infection important chez les patients infectés par le VIH-1 sous traitement anti-rétroviral (HAART) [394]. Nos observations impliquent donc que le déséquilibre observé au niveau de ces deux cytokines a lieu tôt au cours de l'infection. En effet, l'augmentation des niveaux sériques d'IL-18 ainsi que la réduction de son antagoniste (observée lors de notre étude) ont lieu dans les stades précoces de l'infection [395]. Les études menées sur l'IL-18BP ont démontré que sa production est induite dans l'organisme comme un rétro-contrôle négatif en réponse à une augmentation de la production d'IL-18 afin de contrôler les effets biologiques pro-inflammatoires de cette dernière. Cette induction d'IL-18BP est en partie due à la production d'IFN-γ par les cellules T et les cellules NK en réponse à l'IL-18. L'IFN-γ est un stimulus qui permet de produire des niveaux importants d'IL-18BP [396]. La réduction dans la production d'IL-18BP lors de l'infection semble être indépendante de la production d'IFN-γ lors de nos expériences *in vitro* car les macrophages sont reconnus pour ne pas produire cet interféron. De plus, nos données démontrent que l'IL-18 peut aussi directement inhiber la production de son antagoniste au niveau des macrophages humains. Une baisse dans la production d'IFN-γ circulants a été observée chez les patients infectés par le VIH-1 [397]. Une baisse dans la production des cytokines de type Th1, pour lesquelles l'IFN-γ est un prototype, a été impliquée dans l'immunopathogénèse associée au SIDA [276]. Bien que l'IL-18 soit un inducteur puissant d'IFN-γ au niveau des cellules T et des cellules NK, cet effet n'est observable que lorsqu'on combine l'IL-18 avec d'autres cytokines comme l'IL-12, l'IL-15 ou l'IL-21. Comme les niveaux de ces trois cytokines sont considérablement réduits chez les personnes infectées, il n'est donc pas surprenant que la production d'IFN-γ soit faible chez ces mêmes patients [338, 398, 399]. De plus, des cytokines immuno-suppressives comme le TGF-β et l'IL-10 sont reconnues pour inhiber la production d'IFN-γ en

réponse à l'IL-18. Les concentrations circulantes de ces cytokines sont d'ailleurs élevées chez les patients infectés par le VIH-1 [389, 398, 400-402]. Nous avons, lors de notre étude, démontré que le traitement de macrophages avec ces cytokines réduisait la production d'IL-18BP et ce d'une façon indépendante à l'IFN-γ. Ainsi, il semble que le milieu environnant les macrophages lors de l'infection *in vivo* peut aussi contribuer, en parallèle à l'infection, à une réduction dans la production d'IL-18BP chez les patients.
Des résultats conflictuels ont été obtenus concernant l'effet de l'IL-18 sur la réplication du VIH-1 *in vitro* [403]. Cette cytokine stimule en effet la réplication du VIH-1 *in vitro* dans des lignées cellulaires de type monocytaires et de type T alors que cette augmentation n'est pas observée au niveau des cellules primaires [404, 405]. Cependant, une étude a reporté que l'IL-18 inhibait la réplication des souches VIH-1 M- et T-tropiques au niveau des PBMC totaux, et que cet effet inhibiteur était du à une augmentation dans la production d'IFN-γ [406]. Au niveau des PBMC, cet effet inhibiteur pourrait avoir lieu à cause de la production d'IFN-γ par les cellules NK. Cependant, l'IL-18 augmente la réplication du VIH-1 dans les cellules T $CD4^+$ isolées, dans des lignées cellulaires de type T de patients infectés par le VIH-1, et dans des cellules monocytiques infectées par le VIH-1 au niveau desquelles l'IL-18 seule devrait induire la production d'IFN-γ. Comme mentionné plus haut, l'IL-18 à elle seule n'induit que faiblement la production d'IFN-γ et a besoin de la coopération d'autres cytokines. Sachant donc que les concentrations des cytokines collaboratrices sont réduites et que les concentrations de TGF-β et d'IL-10 sont élevées chez les patients, il ne serait donc pas surprenant que l'IL-18 augmente la réplication du VIH-1 lors de l'infection de PBMC *in vivo*, ceci étant du principalement à une réduction des niveaux d'IFN-γ circulants. Une étude a d'ailleurs mis en relation la progression vers le SIDA et les niveaux élevés d'IL-18 et de son antagoniste chez les patients infectés [390, 407, 408].

L'IL-18 semble aussi avoir un rôle important au-delà du système immunitaire chez les patients infectés par le VIH-1 (voir chapitre 3 et Figure 13) [409]. Il a été documenté que cette cytokine jouait un rôle clef dans la déplétion des graisses sous-cutanées au niveau des membres et du visage des patients sous traitement anti-rétroviral [410]. Cette fonte des graisses est un signe caractéristique de la lipodystrophie associée à

ce traitement [411-413]. Cette cytokine est également susceptible d'être impliquée dans l'incidence élevée des plaques athérosclérotiques, à la résistance systémique à l'insuline et donc au développement du diabète de type 2 chez ces patients [414-420]. De plus, une augmentation de la production de cette cytokine dans le cerveau en réponse au stress pourrait mener à des disfonctionnements cognitifs et moteurs qui aboutiront au développement d'une démence associée à l'infection par le VIH-1, un phénomène observé chez ces patients [421-423].

### 13-2 Discussion sur les études concernant l'IL-21

Nos études portant sur l'IL-21 ont permis de montrer que les niveaux sériques d'IL-21 sont drastiquement réduits chez les patients infectés par le VIH-1 (Figure 13). Nous avons étudié sa régulation au niveau d'une très large cohorte de patients comprenant des patient au stade de la primo-infection, des patients chroniquements infectés avec ou sans traitement anti-rétroviral ainsi que différentes categories de patients non-progresseurs à long termes (LTNP). Nous avons aussi étudié l'expression de cette cytokine en réalisant une étude longitudinale sur des patients en primo-infection et chroniquement infectés avec ou sans traitement anti-rétroviral. Nos résultats démontrent pour la première fois que la production d'IL-21 est compromise dans la phase précoce de l'infection. Les sérums de tous nos différents groupes de patients ont démontré des concentrations réduites de cette cytokine par rapport aux sérums de patients non infectés par le VIH-1. Seuls les patients contrôleurs élites ne possèdaient pas de faibles niveaux d'IL-21 cicrulante au niveau de leurs sérums. Notre étude longitudinale chez des patients avec et sans traitement anti-rétroviral a révélée que le traitement a tendance à augmenter les concentrations sériques d'IL-21 bien que cette restauration soit partielle. D'une façon consistante avec nos résultats, le traitement anti-rétroviral permet aussi d'augmenter les concentrations d'IL-2 et d'IL-15 chez les patients infectés par le VIH-1 [424-427].

Une forte corrélation entre le taux de cellules T $CD4^+$ et les concentrations d'IL-21 sériques a été observée chez tous les différents groupes de patients. Cette corrélation a

aussi été observée lorsque les différents groupes formant la cohorte ont été regroupés d'une façon indépendante à leur stade clinique et leur traitement anti-rétroviral. Ces résultats suggèrent que les concentrations d'IL-21 circulante pourraient être considérées comme un biomarqueur fiable nous renseignant sur la perte des cellules T $CD4^+$, la progression de la maladie, et la reconstitution immunitaire chez les patients infectés par le VIH-1. Ce marqueur pourrait être d'une grande utilité lorsque les chercheurs et les cliniciens ne peuvent avoir accès qu'à une quantité limitée de matériel biologique ou lorsqu'ils ne possèdent seulement que les sérums de patients. Ces résultats indiquent aussi que la déplétion des cellules T $CD4^+$, qui est reconnue comme la caractéristique principale du développement du SIDA, contribue à la baisse de la concentration circulante de cette cytokine chez les patients infectés.

Lors de cette étude, nous avons démontré que les cellules T $CD4^+$ des patients infectés ont une capacité réduite à produire l'IL-21 par rapport aux cellules provenant de patients non infectés. De plus, la quantité des différents sous-types de cellules T $CD4^+$ spécifiques au VIH-1 et produisant de l'IL-21 ($CD27^+CD45RA^-CCR7^+$, $CD27^+CD45RA^-CCR7^-$ and $CD27^-CD45RA^-CCR7^-$) est significativement réduite par rapport aux mêmes sous-types de cellules qui sont spécifiques au Cytomégalovirus humain (HCMV). La production d'IFN-γ au niveau de ces cellules spécifiques au VIH-1 n'est cependant pas différente de celle des cellules spécifiques au HCMV. Nos données suggèrent donc que l'infection par le VIH-1 cause une déplétion des cellules produisant de l'IL-21 qui sont spécifiques au VIH-1. Bien que nos données obtenues lors d'expériences *in vitro* sur des cellules T $CD4^+$ infectées par le VIH-1 démontrent que l'infection n'affecte pas la production d'IFN-γ, des études *ex vivo* réalisées sur des PBMC de patients infectés par le VIH-1 ont révélé que la fréquence des cellules T $CD4^+$ spécifiques au HCMV (de phénotype $CD27^-CD45RA^-CCR7^-$ et produisant des cytokines) était réduite chez ces patients par rapport aux donneurs sains. Cette différence pourrait être attribuée au fait que *in vivo*, beaucoup de facteurs induits par l'infection virale peuvent affecter la production de ces cytokines. Il est important de noter que le sous-type $CD27^-CD45RA^-CCR7^-$ de cellules T $CD4^+$ est complétement différencié et produit des quantités maximales d'IFN-γ [341]. D'autres études seraient nécessaires afin

de déterminer ce qui cause vraiment une réduction de la fréquence des cellules T $CD4^+$ spécifiques au VIH-1 chez les patients.

Nous avons démontré pour la première fois dans cette étude que l'infection *in vitro* de cellules T $CD4^+$ humaines réduit leur capacité à produire de l'IL-21. Ceci explique en partie la baisse dans la capacité des cellules T $CD4^+$ de patients infectés par le VIH-1 à produire cette cytokine. Cependant, toutes les cellules T $CD4^+$ présentes dans l'organisme des patients infectés ne sont pas infectées par le virus [428]. Il est donc fortement probable qu'une ou plusieurs cytokines immunosuppressives présentes en grande quantité dans leurs organisme, comme l'IL-10 et le TGF-β [322, 350], pourraient aussi induire une réduction dans la sécrétion de l'IL-21 au niveau de ces cellules. Il est claire que d'autres études à ce sujet devraient être considérées afin d'investiguer le rôle du réseau de cytokines présent chez les patients sur la production de l'IL-21.

Une étude convaincante a démontré le rôle clef du facteur de transcription c-Maf dans la transcription de l'IL-21 [80]. En effet, les souris KO pour ce facteur de transcription ont une réduction drastique des concentrations d'IL-21 circulantes [80]. Le facteur de transcription c-Maf est l'homologue cellulaire de la protéine oncogène virale v-Maf et représente l'un des premiers facteurs identifiés étant impliqué dans la production des cytokines de type Th2. Une augmentation de la présence de ce facteur de transcription est d'ailleurs reconnue pour augmenter la production d'IL-21 au niveau des cellules T $CD4^+$ [80, 429]. Par conséquent, nous avons chercher à déterminer si la baisse dans la production de l'IL-21 lors de l'infection *in vitro* était en relation avec une éventuelle régulation de ce facteur de transcription. Nos expériences ont en effet démontré que l'infection de PBMC et de cellules T $CD4^+$ isolées *in vitro* résulte sur une réduction de la production de c-Maf. Cette réduction a lieu uniquement au niveau des cellules infectées produisant du virus et non dans les cellules non infectées. Nos résultats sont de plus renforcés par le fait que l'inhibition de c-Maf par un siRNA entraîne une réduction de la production d'IL-21 et d'IL-4, qui est reconnue comme une cytokine de type Th2 étant positivement régulée par ce facteur de transcription [429]. Il semble donc que la réplication virale soit nécessaire pour permettre une baisse de la présence du facteur de transcription c-Maf, résultant ainsi sur une réduction de la production d'IL-21. En effet, nos résultats démontrent aussi que l'infection de ces cellules par un virus inactivé par

rayons ultra-violets ou par la chaleur ne permet pas de réduire la production de cette cytokine ainsi que celle de ce facteur de transcription (résultats non montrés). Ces résultats on donc permis de révéler un des mécanismes moléculaires permettant la réduction de la production de l'IL-21 au niveau des cellules T CD4$^+$ infectées par le VIH-1.

Une baisse de la capacité de survie *ex vivo* des cellules immunes des patients infectés par le VIH-1 représente un fait très bien documenté [314, 430, 431]. La déplétion des cellules T CD4$^+$, qui sont la cible principale du VIH-1, représente l'observation caractéristiques du SIDA et la restauration complète de ce compartiment cellulaire représente un véritable challenge chez les patients [430, 432]. Le traitement anti-rétroviral (HAART) est reconnu pour être très efficace en ce qui concerne l'arrêt de la réplication virale et la diminution de la charge virale mais est nettement moins efficace en ce qui concerne la restauration des taux de cellules T CD4$^+$ à leurs niveaux physiologiques normaux. De ce fait, les scientifiques tentent donc de trouver des moyens de pallier à ce problème en utilisant des thérapies basées sur des cytokines telles que l'IL-2, l'IL-7 et l'IL-15, qui ont un rôle clef sur l'homéostasie des cellules T (voir chapitre 7) [373, 377, 433-435]. Nos résultats démontrent d'ailleurs que l'IL-21 possède la capacité d'empêcher la mort des cellules T CD4$^+$ lors de cultures *ex vivo*. Cet effet positif sur la survie des cellules T CD4$^+$ et CD8$^+$ a déjà été démontré ultérieurement [436]. Ces études ont démontré que l'IL-21 active la PI-3K, augmente l'expression de la protéine Bcl-2, empêche la réduction de la taille des cellules et une mort spontanée lors de cultures *ex vivo*. Nos résultats démontrent de plus que cet effet de survie sur les cellules T CD4$^+$ de patients infectés par le VIH-1 est plus important que celui observé sur les cellules patients non infectés. L'IL-21 a permis d'augmenter d'un facteur 6 le nombre de cellules T CD4$^+$ vivantes au niveau des patients infectés alors que seulement d'un facteur 3 pour les cellules de donneurs sains. Ceci pourrait d'ailleurs être dû à une augmentation de l'expression de l'IL-21R à la surface des cellules T CD4$^+$ de patients. Ces résultats suggèrent qu'une réduction de la survie de ces cellules *ex vivo* pourraient être une conséquence de la baisse de la production de cette cytokine. Cette cytokine pourrait donc représenter un outil thérapeutique approprié pour restaurer la survie des

cellules T $CD4^+$ ainsi que d'autres lymphocytes, et finalement augmenter la reconstitution immune chez les patients.

Plusieurs études ont démontré les différents effets biologiques de l'IL-21 permettant de renforcer les fonctions effectrices des cellules immunes. L'IL-21 augmente le potentiel cytotoxique des cellules T $CD8^+$, notamment en induisant l'expression de perforine [155]. Ainsi, des faibles niveaux d'IL-21 chez les patients infectés par le VIH-1 pourraient résulter sur des fonctions effectrices de ces cellules qui seraient compromises. En effet, une baisse de l'expression de perforine au niveau des cellules T $CD8^+$ est observée chez les patients [341]. Plusieurs études futures seront nécessaires afin de comprendre et d'apprécier de façon plus concrète les conséquences de la réduction de cette cytokine chez les patients, son impact sur la réponse immunitaire antivirale et la progression de la maladie. Des faibles niveaux d'IL-21 pourraient donc affecter la survie ainsi que la prolifération et la fonction de plusieurs cellules immunes comme les cellules T cytotoxiques, les cellules NK et bien d'autres compartiments cellulaires. Nous avons d'ailleurs démontré que des concentrations élevées d'IL-21 sont associées avec une haute fréquence de cellules T $CD8^+$ ainsi qu'une meilleure fonctionnalité de ces cellules chez les patients infectés par le VIH-1. Ainsi, une déficience au niveau de cette cytokine pourrait affecter la capacité d'un individu à contrôler l'infection. En effet, des études *in vivo* récentes ont montré que l'IL-21 est une cytokine indispensable pour contrôler l'infection virale chronique (voir chapitre 2-6) [230-232]. Une production compromise de cette cytokine pourrait donc être une des raisons qui empêche l'homme de contrôler l'infection par le VIH-1 et le développement du SIDA.

Lors de nos études, nous nous sommes intéréssés à la capacité de l'IL-21 à moduler les activités cytotoxiques et sécrétrices des cellules NK humaines (Figure 13). Nous avons démontré pour la première fois que tous les sous-types majeurs de cellules NK ($CD56^{bright}CD16^+$, $CD56^{dim}CD16^+$, $CD56^{bright}CD16^-$, $CD56^-CD16^+$) exprimaient de façon constitutive des niveaux équivalents d'IL-21R. De plus, notre étude a permis de montrer que l'expression de l'IL-21R n'est aucunement altérée chez les patients infectés par le VIH-1 quel que soit leur stade clinique et ce pour tous les différents sous-types de cellules NK. Par conséquent, ces données suggèrent que tous les sous-types de cellules

NK peuvent répondre de façon similaire à de l'IL-21 recombinante exogène. Ces données sont d'ailleurs consistantes avec d'autres études qui ont reporté une expression équivalente du récepteur à la surface des sous-types $CD3^-CD56^{bright}$ et $CD3^-CD56^{dim}$ [437].

Nous avons démontré lors de cette étude que l'IL-21 augmente la cytotoxicité médiée par les cellules NK contre des cellules cibles (CEM). L'IL-21 augmente l'expression de marqueurs de fonctionnalités cytotoxiques comme la perforine, le marqueur de dégranulation CD107a (LAMP-1) et l'IFN-γ. Un effet additif a été observé lorsque l'IL-21 a été combinée à de l'IL-2 ou de l'IL-15 concernant la cytotoxicité des cellules NK. Lorsque que nous avons incubé des cellules cibles exprimant de façon stable les gp120/gp41 du VIH-1 avec de sérums de patients infectés par le VIH-1, nous avons démontré que l'IL-21 augmente la cytotoxicité cellulaire dépendante des anticorps (ADCC) médiée par les cellules NK. L'IL-21 possède la capacité d'augmenter le mécanisme d'ADCC à un niveau similaire à d'autres cytokines comme l'IL-2 ou l'IL-15. Aucun effet additif sur l'ADCC a été observé lorsque ces cytokines ont été utilisées en combinaison, ceci étant sûrement dû à une augmentation parallèle de la lyse directe des cellules cibles. Lors de l'ADCC, les cellules NK lysent leurs cibles en utilisant des anticorps qui se lient au niveau de leur partie Fc à un récepteur de faible affinité présent sur ces cellules appelé CD16 (FcγRIIIa) [438]. Nos études ont démontré que l'IL-21 n'affecte pas l'expression du CD16 à la surface des cellules NK tant au niveau des cellules de patients que des cellules de donneurs sains (résultats non montrés). Plusieurs études avaient démontré ultérieurement que l'IL-21 augmente la lyse directe ainsi que l'ADCC de cellules NK humaines et murines [439-442].

Il est reconnu que, après liaison à l'IL-21R, l'IL-21 permet la phosphorylation et donc l'activation la protéine STAT-3 dans les cellules T et les cellules NK [443]. Nos résultats expérimentaux confirment ceci et démontrent de plus que la signalisation médiée par l'IL-21 a lieu en activant les protéines ERK-1, ERK-2, p38 et Akt au niveau des cellules NK. Nous n'avons cependant pas observé d'activation des protéines JNK et Vav. De plus, nous avons démontré le lien entre ces signalisations et les fonctions effectrices des cellules NK. Nous montrons que la cucurbitacine, un inhibiteur pharmacologique de la phosphorylation de STAT-3, inhibe complètement

l'augmentation des fonctions effectrices médiée par l'IL-21 comme la cytotoxicité, la sécrétion d'IFN-γ, la dégranulation et l'expression de perforine. Les inhibiteurs de la PI-3K, de Akt, des ERK et de la p38 ont tous été capable d'inhiber les fonctions des cellules NK sauf la capacité de dégranulation de ces dernières (résultats non montrés).

Contrairement aux cytokines appartenant à la même famille, l'IL-21 n'augmente pas à elle seule la prolifération des cellules NK. Lorsqu'utilisée seule, l'IL-21 n'a pas augmenté la capture de thymidine tritiée au niveau des cultures *in vitro*. Cependant, cette cytokine augmente la synthèse d'ADN et la prolifération de ces cellules en combinaison avec d'autres cytokines comme l'IL-2 et l'IL-15. Des résultats similaires concernant son effet sur la prolifération ont pu être observés avec les cellules T $CD8^+$ [91, 149, 444]. Ainsi, nos résultats diffèrent de ceux obtenus lors de différentes études qui ont reporté un effet inhibiteur ou activateur de l'IL-21 utilisée seule sur la prolifération de ces cellules [165, 170]. Nous montrons lors de notre étude, et ce pour la première fois, que l'IL-21 augmente l'expression des protéines anti-apoptotiques Bcl-2 et Bcl-$X_L$ au niveau des cellules NK. Elle augmente de ce fait la viabilité de ces dernières lors de cultures avec des conditions sub-optimales de croissance (faible concentration en sérum foetal). Cette cytokine était déjà connue pour avoir cet effet au niveau des cellules T murines ainsi que pour augmenter leur granulosité et leur taille [436]. Lors de nos expériences, nous avons seulement observé une augmentation de la granulosité des cellules NK humaines mais aucune augmentation au niveau de leur taille a été observée. En augmentant la viabilité des cellules NK, l'IL-21 contribue donc à leur prolifération induite par d'autres cytokines. Ces effets de survie de l'IL-21 sont donc similaires à ceux observés au niveau des cellules T.

Lors de notre étude, nous avons aussi voulu déterminer si l'IL-21 pouvait améliorer les capacités fonctionnelles des cellules NK de patients infectés lors d'expériences *ex vivo*. Nous avons démontré que l'IL-21 augmente de façon significative la cytotoxicité, la dégranulation, la survie ainsi que l'ADCC médiés par les cellules NK de ces patients. Nos résultats sont donc en accord avec ceux obtenus par Strbo et al. qui ont reporté un effet activateur de cette cytokine sur les fonctions effectrices médiées par ces cellules chez les patients infectés par le VIH-1 [443]. Ce groupe de recherche a d'ailleurs documenté une effet positif de cette cytokine sur les cellules T $CD8^+$ de patients infectés

par le VIH-1 [155]. Lors de leur étude utilisant des patients avec une charge virale inférieure à 50 copies/ml et un taux de cellules T $CD4^+$ supérieur à 200 cellules/µl de sang, ils ont montré que le traitement des cellules T $CD8^+$ avec l'IL-21 résultait sur une augmentation d'expression de la perforine, spécialement au niveau des sous-types mémoires et effecteurs. Cependant, l'IL-21 n'a pas augmenté la dégranulation induite par le TCR de ces cellules, ni leur prolifération et leur état général d'activation. D'une manière générale, ces données montrent de façon claire que l'IL-21 est capable d'augmenter les activités fonctionnelles des cellules NK de patients lors d'expériences *ex vivo*. Sachant que les concentrations d'IL-21 sont réduites chez ces patients, ces données sont donc d'une relevance particulière concernant le contrôle de l'infection par le système immunitaire.

Malgré leur effet positif sur l'immunité antivirale, le principal désavantage de l'utilisation des cytokines, ou d'autres modificateurs de la réponse biologique, est leur capacité à augmenter la réplication du VIH-1. L'IL-15 est d'ailleurs reconnue pour avoir un effet plus faible concernant cette augmentation de la réplication virale par rapport à d'autres cytokines comme l'IL-2 ou l'IL-12 [445-447]. L'IL-7 a aussi été démontré comme une cytokine capable d'augmenter la réplication du VIH-1 chez les patients infectés [448]. En comparant trois différentes cytokines qui sont l'IL-2, l'IL-15 et l'IL-21, nous avons obervé que cette dernière cytokine était la cytokine qui activait le moins la réplication du VIH-1. L'IL-21 ne modifie pas l'expression du récepteur et des co-récepteurs permettant l'entrée du virus dans les cellules cibles, et n'active pas la transcription du génome viral à partir du LTR du VIH-1 (résultats non montrés). Cette cytokine n'augmente pas la réplication du VIH-1 lors de l'infection de macrophages humains par une souche M-tropique (résultats non montrés). De plus, nos résultats ont démontré que cette cytokine n'augmente pas la production de virus endogènes lorsqu'on l'ajoute à des cultures *ex vivo* prolongées de PBMC provenant de patients chroniquement infectés virémiques et sans thérapie anti-rétrovirale.

Plusieurs études *in vitro* ont démontré la capacité des cellules NK à supprimer la réplication du VIH-1 lors de co-cultures avec des cellules T $CD4^+$ autologues infectées [262, 449]. Lors de notre étude, nous avons voulu déterminer l'effet de l'IL-21 sur la réplication virale lors de ces cultures. Nous avons démontré que l'IL-21 augmente

l'inhibition de la réplication médiée par les cellules NK lors de co-cultures avec des cellules T $CD4^+$ autologues. En utilisant des anticorps neutralisant dirigés contre la protéine d'adhésion LFA-1, contre la perforine, ou contre l'IFN-γ, nous avons investigué si cette inhibition de la réplication virale avait lieu lors d'un contact entre cellules NK et cellules T $CD4^+$, ou était simplement due à la sécrétion d'IFN-γ par les cellules NK. Nous avons démontré un rôle important de la protéine LFA-1, permettant le contact entre les deux types cellulaires, dans l'inhibition médiée par les cellules NK. Il semble donc qu'un contact cellulaire entre la cellule NK et sa cible soit nécessaire pour permettre cette inhibition. En effet, lors de l'utilisation d'un anticorps contre l'IFN-γ, l'effet suppresseur des cellules NK n'a pas été inhibé, ce qui suggère que ce facteur soluble ne joue en rien sur cette inhibition. LFA-1, en interagissant avec la protéine ICAM-3, est une protéine nécessaire permettant la formation d'une synapse immunologique entre les cellules NK et leurs cibles [450-452]. Cette interaction favorise aussi grandement la capacité des cellules NK à dégranuler [452]. Le fait que l'inhibition de LFA-1, et non celle de la perforine, bloque complétement l'effet suppresseur des cellules NK suggère que l'anticorps anti-LFA-1 inhibe aussi l'infection et la dissémination du virus d'une cellule T $CD4^+$ à une autre cellule T $CD4^+$. En effet, LFA-1 joue un rôle important au niveau de ce type de dissémination du VIH-1 qui représente sûrement un des modes d'infections le plus efficace *in vivo* [453]. Nos résultats *in vitro* ont aussi démontré que l'IL-21 n'a aucun effet sur l'expression de LFA-1 à la surface de cellules NK (résultats non montrés). Cependant, il reste à savoir si l'IL-21 ne pourrait pas causer des changements conformationels permettant d'augmenter l'affinité de cette molécule d'adhésion à son ligand. Finalement, nos résultats de co-cultures *in vitro* ont montré que les cellules NK traitées avec de l'IL-21 pouvaient lyser de façon efficace des cellules T $CD4^+$ autologues infectées par le VIH-1. Ainsi, cette cytokine pourrait être utilisée pour augmenter les fonctions antivirales des cellules NK afin de contrôler la réplication du VIH-1 chez les patients infectés.

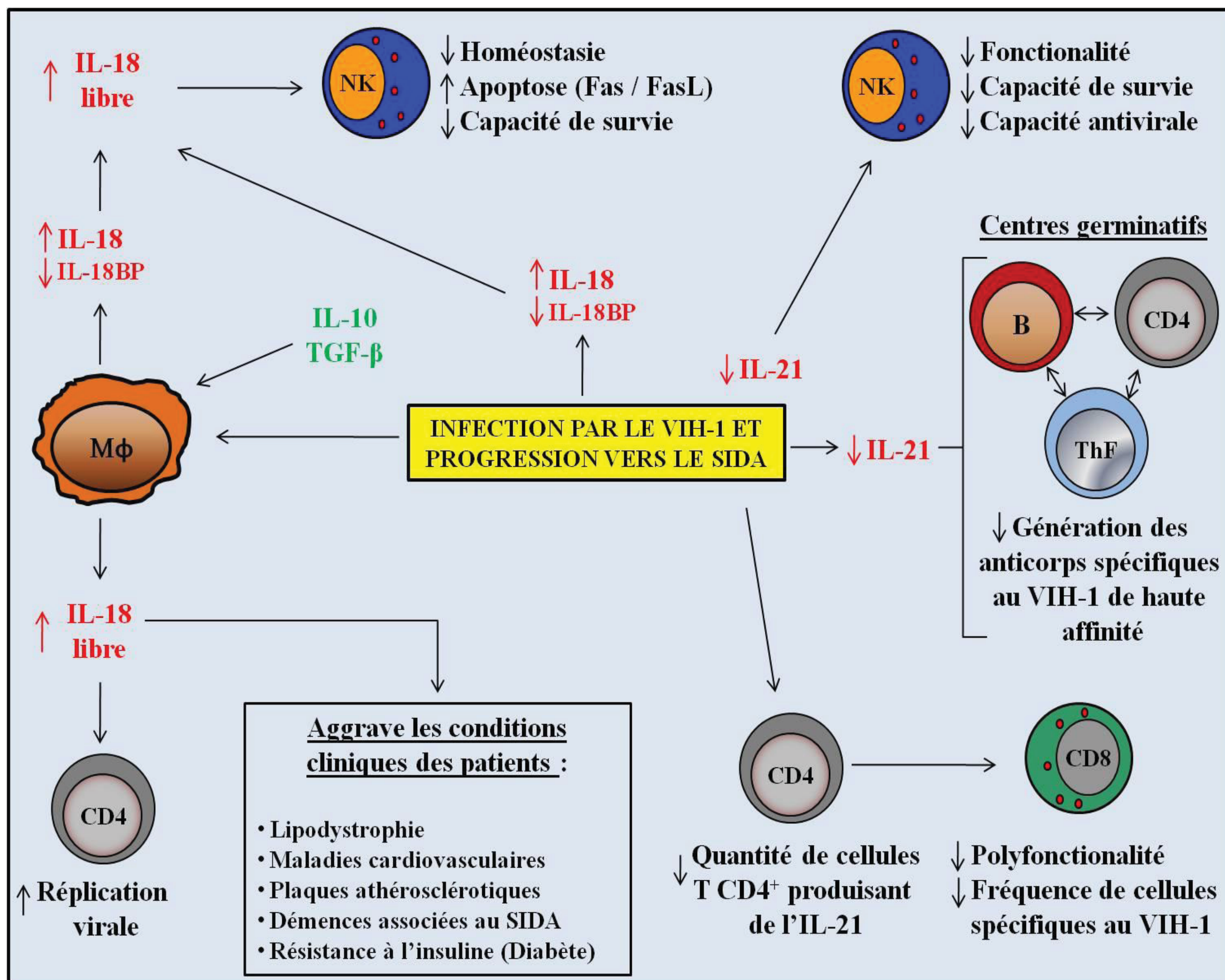


**Figure 13. Schéma récapitulant l'ensemble des découvertes réalisées sur la dérégulation de l'IL-18 et de l'IL-21 lors de l'infection par le VIH-1 et leurs conséquences sur le système immunitaire de l'hôte infecté.**

L'utilisation des cytokines de type I comme l'IL-2, l'IL-7 et l'IL-15 afin d'augmenter les fonctions, la prolifération, et la survie des cellules ayant un rôle dans l'immunité antivirale chez les patients infectés par le VIH-1 a été d'un grand intérêt depuis les dernières décénnies. L'utlilisation potentielle de l'IL-21 dans le traitement des maladies virales est donc à son tour d'un intérêt particulier pour la communauté scientifique. Lors d'une étude réalisée en utilisant un modèle murin d'infection par le HSV-1, Rodrigues et al. ont montré que l'injection d'un plasmide codant pour l'IL-21 augmentait la fréquence des cellules T $CD8^+$ spécifiques au virus même sans la présence d'antigènes viraux, et que la force de la réponse médiée par ces cellules étaient dépendante de la signalisation par leur TCR [454]. L'injection de plasmides codant pour l'IL-15 et l'IL-21 a permis d'augmenter le nombre de cellules T $CD8^+$ spécifiques aux antigènes du HSV-1 qui produisent de l'IFN-γ lors de la phase aigüe de l'infection. Lors de l'infection chronique, le nombre de cellules T $CD8^+$ produisant de l'IFN-γ et du TNF-α a été augmenté lors de l'injection de plasmides codant pour l'IL-15 et l'IL-21 seules ou en combinaison. Leur étude montre aussi une association entre la présence d'antigènes viraux et l'injection de plasmides codant pour ces cytokines permettant une augmentation de la réponse proliférative des cellules T $CD8^+$. De plus, la combinaison de l'ADN codant pour ces cytokines ou la combinaison de cet ADN et des antigènes viraux augmente la prolifération et la polyfonctionnalité des cellules T $CD8^+$. Ces données suggèrent que ces deux cytokines peuvent contribuer à augmenter la réponse immunitaire antivirale contre l'infection par le HSV-1, et à renforcer leur capacité à surpasser l'absence d'antigènes viraux pour médier les fonctions effectrices des cellules T $CD8^+$.

Lors d'une autre étude, l'injection intravasculaire d'un ADN plasmidique codant pour la glycoprotéine B du HSV-1, ainsi que celle d'un plasmide codant pour l'IL-21, a été capable d'élever le taux de survie des souris infectées par le HSV-1 [455]. Les lymphotytes T cytotoxiques spécifiques à la glycoprotéine B ainsi que la production d'anticorps neutralisant contre le virus ont été augmentés.

Des études réalisées sur des cellules T $CD4^+$ spécifiques au HCMV ont démontré que la stimulation du sous-type $CD45RA^+CD27^-CCR7^-$ avec leurs peptides appropriés ainsi que par l'IL-21 a permis d'induire leur expansion clonale [444]. De plus, l'IL-21 a

permis de changer leur phénotype vers un phénotype CD45RO et a permis de promouvoir l'expression du récepteur CCR7, alors que leurs fonctions effectrices sont restées intact. D'une manière similaire, des études récentes ont montré que la stimulation antigène-spécifique *in vitro* par des peptides du HCMV en combinaison avec de l'IL-21 permet d'induire la ré-expression du CCR7 sur les cellules T $CD8^+$ spécifiques au HCMV [456]. Ces cellules qui ré-expriment le CCR7 exprimeront de plus le CD62L et pourront réagir de façon spécifique à une stimulation par des chimiokines. En effet, ces cellules ont été capable de migrer en présence d'un gradient de chimiokines lors d'expériences classiques de migration *in vitro*. Ces données suggèrent que la stimulation antigènique combinée avec le traitement par l'IL-21 peut induire la ré-expression fonctionelle du CCR7, autorisant ainsi les cellules T $CD8^+$ à migrer au niveau des sites inflammatoires critiques lors des infections virales. Par conséquent, l'IL-21 pourrait non seulement augmenter l'exécution directe des fonctions effectrices de ces cellules, mais pourrait aussi aider au contrôle de la réplication des virus dans une situation de ré-infection ou de ré-activation de virus latents.

Lors d'études *in vivo*, Bolesta et al. ont démontré qu'un plasmide codant pour l'IL-21 seule ou en combinaison avec celui codant pour l'IL-15 étaient capable de réguler la réponse immunitaire contre la glycoprotéine d'enveloppe (Env) du VIH-1 [439]. Des souris ont été injectées par un vecteur exprimant les protéines Env modifiées contenant des mutations au niveau de la région C-terminale. Au niveau de ce vecteur, les régions divergentes et variables V1, V2 et V3 de la souche CXCR4-tropique (HxB2) ont été remplacées par la région *Env* de la souche virale double tropique 89.6. Ce virus recombinant de la vaccine exprimait donc la glycoprotéine Env du virus 89.6 (vBD3). En injectant ce virus recombinant dans les souris, ils ont remarqué que l'injection d'un plasmide codant pour l'IL-21 a permis de fournir une résistance soutenue à la réplication et à la transmission du virus. Les plasmides codant pour l'IL-21 et l'IL-15 ont agit de façon synergétique pour augmenter la réponse immunitaire mémoire induite lors du challenge par le vecteur recombinant vBD3. La synergie entre ces deux cytokines a permis de promouvoir l'expansion des cellules T $CD8^+$ mémoires spécifiques aux glycoprotéines d'enveloppes. Le traitement par l'IL-21 et l'IL-15 a résulté sur l'augmentation des fonctions effectrices des cellules T $CD8^+$, permettant ainsi une

protection efficace contre le vBD3. Cette protection a semblé être partiellement indépendante de l'aide des cellules T $CD4^{+}$. De plus, ces deux cytokines ont été capable d'augmenter l'ADCC et la lyse médiée par le complément (CDCC) contre des cellules cibles exprimant les glycoprotéines d'enveloppes en augmentant le niveau de production d'anticorps neutralisants spécifiques à ces protéines.

Plusieurs études pré-cliniques ont aussi démontré le rôle potentiel de l'IL-21 exogène dans la réponse anti-tumorale et ce au niveau de différents modèles *in vivo* de cancers (voir chapitre 2-6). De plus, plusieurs études *in vitro* ont démontré que l'IL-21 pouvait directement induire l'apoptose des lymphômes de cellules B divers et des cellules leucémiques lymphocytiques chroniques. Ces données prometteuses ont permis aux chercheurs et aux cliniciens de commencer des essais cliniques ayant pour but d'utiliser l'IL-21 pour traiter différents types de cancers. A ce jour, trois essais cliniques de phase I et II utilisant l'IL-21 recombinante ont été complétés concernant les mélanomes, les carcinomes de cellules rénales et les lymphômes B non-Hodgkiniens [457-459]. Ces études cliniques ont fournit des informations précieuses sur l'efficacité et la tolérance de ce traitement chez des patients ayant atteints des stades avancés de cancers. De plus, des essais cliniques basés sur l'utilisation de l'IL-21 seule ou en combinaison avec d'autres agents thérapeutiques sont en cours concernant le traitement d'une variété de cancers. D'une manière générale, l'ensemble de ces études ont permis de démontrer que des cycles d'injection répétés d'IL-21 exogène sont bien tolérés par le corps et que l'administration de cette cytokine ne semble pas déclencher de réaction auto-immune.

## 14- Conclusions

Bien que le VIH-1 ait été étudié intensivement depuis sa découverte en 1983, les mécanismes moléculaires résultant sur une déficience du système immunitaire ainsi que sa restauration sont toujours d'intérêt. Les moyens permettant d'élaborer de nouvelles stratégies thérapeutiques, permettant de promouvoir la réponse immunitaire antivirale, sont toujours sous investigations par la communauté scientifique.
La difficulté concernant la compréhension de ces mécanismes réside dans la complexité incroyable existant entre les facteurs viraux agissant lors de l'infection, les mécanismes immunitaires impliqués dans son contrôle, et spécialement l'immunité médiée par les cytokines. Le développement de souches virales résistantes au traitement ainsi que l'incapacité du traitement à éradiquer complétement le virus de l'organisme soutiennent l'idée et la nécesssité d'un traitement supplémentaire chez ces patients. Ceci aiderait le traitement antiviral à restaurer le système immunitaire de l'hôte infecté pour éliminer totalement le virus. C'est donc par la compréhension de la biologie des cytokines et des mécanismes résultant sur leur dérégulation lors de l'infection que de nouvelles stratégies thréapeutiques pourront être développées. En comprenant comment le virus perturbe le réseau cytokinique de l'hôte infecté, nous pourrons mieux comprendre comment la réponse immunitaire antivirale fonctionne et comment la renforcer pour lutter contre l'infection. Cependant, ces stratégies thérapeutiques se doivent d'être mesurée et fortement contrôlée, car il est bien reconnu de nos jours que les cytokines peuvent exhiber des effets complétement contraires à ceux escomptés si elles sont utilisées d'une manière non appropriée.

Lors de ces études, nous avons finalement pu répondre aux questions que nous nous étions posées. Nous avons exploré la régulation de l'IL-18 et de son antagoniste naturel l'IL-18BP chez les patients infectés par le VIH-1 (Figure 13). Nous avons aussi pu étudier l'impact des niveaux élevés d'IL-18 et du déséquilibre existant avec son antagoniste sur l'homéostasie et la fonctionnalité des cellules NK chez les patients infectés par le VIH-1. Nous avons été capable de mettre en relation l'importance de cette dérégulation sur la réplication du VIH-1 et sur l'immnunopathogénèse associée à

l'infection par le VIH 1. Par ses propriétés pro-inflammatoires et immunostimulantes, l'IL-18 est capable de protéger l'hôte contre les infections virales et bactériennes. En induisant la production d'IFN-γ, elle peut induire un état antiviral dans les cellules hôtes et favoriser le développement d'une réponse antivirale de type Th1. Cependant, la dualité de cette cytokine réside dans sa capacité à promouvoir des réponses de type Th2 en présence d'un environnement cytokinique particulier. Sachant que l'infection par le VIH-1 est accompagnée d'une perturbation drastique du réseau de cytokines produites, la capacité de l'IL-18 à induire la sécrétion d'IFN-γ se trouve affaiblie, ce qui inhibera la production d'IL-18BP et permettra le développement d'une réponse Th2. Le déséquilibre entre l'IL-18 et l'IL-18BP chez les patients infectés peut aussi perturber la réponse antivirale médiée par les cellules NK ou promouvoir la destruction des tissus médiée par la voie Fas/FasL. De plus, l'IL-18 augmente la réplication virale au niveau des cellules T $CD4^+$ infectées. Ainsi, les concentrations élevées d'IL-18 chez les patients semblent agir comme un inconvénient plutôt qu'un avantage.

Les concentrations élevées d'IL-18 chez les personnes infectées par le VIH-1 sont aussi susceptibles de jouer un rôle dans les conditions cliniques associées à l'infection et au traitement anti-rétroviral. Par conséquent, sa neutralisation pourrait représenter une stratégie immunothérapeutique utile et appropriée chez ces patients. Elle pourrait retarder la progression du SIDA et améliorer le système immunitaire des personnes infectées. Afin d'améliorer les conditions de ces patients, les effets néfastes de l'IL-18 pourraient être neutralisés en utilisant son inhibiteur naturel, soit de l'IL-18BP exogène, afin de moduler l'activité de l'IL-18 *in vivo* à des niveaux souhaitables.

Nos études sur l'IL-21 ont permis de comprendre comment l'IL-21 était régulée chez les patients infectés par le VIH-1 (Figure 13). Nous avons pu mettre en évidence, lors de nos études *in vitro*, un des mécanismes moléculaires permettant de réduire la production d'IL-21 au niveau des cellules T $CD4^+$. Nous avons de plus pu mettre en relation les faibles niveaux d'IL-21 observables chez les patients infectés et leur impact sur l'homéostasie des cellules T $CD4^+$, des cellules T $CD8^+$, et des cellules Natural Killer. Nos études ont permis d'oberver l'effet de l'IL-21 exogène sur les fonctions effectrices des cellules du système immunitaire chez les patients infectés par le VIH-1 comme les

cellules Natural Killer et les cellules T $CD8^+$ cytotoxiques. Ces études permettent donc de fournir des informations utiles concernant l'utilisation potentielle de l'IL-21 en tant qu'agent immunothérapeutique. Du fait que cette cytokine possède un rôle pléïotropique sur le système immunitaire, le traitement des patients infectés par le VIH-1 avec l'IL-21 pourrait permettre d'augmenter la réponse antivirale médiée par les lymphocytes T cytotoxiques et les cellules Natural Killer ainsi que la réponse humorale médiée par les cellules B , qui permettra la production d'anticorps neutralisants anti-VIH efficaces. Par conséquent, cette cytokine semble représenter un candidat de première classe pour le traitement des patients souffrant du SIDA ou d'infections virales chroniques d'une manière générale. Dans le cas des patients infectés par le VIH-1 qui développent le SIDA, le traitement par l'IL-21 pourrait être utilisé afin de restaurer les concentrations physiologiques de cette cytokine, afin d'augmenter la réponse antivirale, et finalement aider à la reconstitution des différentes composantes du système immunitaire. Sachant que l'IL-21 est une cytokine qui semble être bien tolérée par l'organisme par rapport à d'autres cytokines, l'utilisation thérapeutique de cette dernière dans le traitement des patients infectés par le VIH-1, ou bien en tant qu'adjuvant vaccinal, devrait être sérieusement considérée dans un futur proche.

## 15- Perspectives

Plusieures perspectives découlent des travaux realisés pendant ma thèse de doctorat ainsi que ceux réalisés par d'autres équipes travaillant sur ces cytokines.

Par exemple, aucune information n'est disponible à ce jour sur l'expression de l'IL-18R chez les patients infectés par le VIH-1 ainsi que sur la bonne fonctionnalité de ce dernier lors d'une signalisation par l'IL-18. L'effet néfaste des niveaux élevés de l'IL-18 sur la réponse immune antivirale ainsi que leurs influences sur les conditions cliniques des patients suggèrent fortement des essais cliniques ciblant sa neutralisation chez les personnes infectées par le VIH-1. L'inhibition de l'IL-18 pourrait être réalisée en utilisant de l'IL-18BP exogène.

A la vue des études présentées dans cette thèse, il semble important d'étudier l'expression et la régulation de l'IL-21 dans d'autres maladies virales chroniques comme celles induites par le virus de l'hépatite C et le virus de l'influenza, ou bien lors d'infections par des virus herpès.

Afin d'être capable d'augmenter ou de neutraliser les effets biologiques de l'IL-21 lors d'infections, il est nécessaire de comprendre à quel(s) stage(s) et dans quel(s) contexte(s) la signalisation médiée par l'IL-21 est nécessaire.

Bien que cela puisse paraître surprenant, les sites de production *in vivo* de l'IL-21 ne sont toujours pas définis. L'utilisation de souris contenant un gène rapporteur pour cette cytokine permettra de répondre d'une façon tissu-spécifique à cette question. Ceci permettra de connaître l'expression physiologique de l'IL-21 ainsi que les mécanismes contrôlant les niveaux de cette cytokine. Il semble donc important de mieux comprendre et de développer des façons de contrôler l'expression de l'IL-21 et de son récepteur. Ceci passera donc par une meilleure compréhension des mécanismes de signalisation médiés par l'IL-21.

Une bonne compréhension de l'effet de l'IL-21 sur les différents compartiments du système immunitaire aura un impact majeur sur les stratégies thérapeutiques au niveau clinique. Les effets pléïotropiques de l'IL-21 sur les différentes cellules du système immunitaire ayant un rôle dans la réponse anti-tumorale et anti-microbienne suggèrent fortement l'utilisation potentielle de cette cytokine pour des thérapies et la vaccination.

## 16- Références bibliographiques

[1] Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA, et al. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. N Engl J Med. 1981;305:1425-31.

[2] Groopman J, Gottlieb M. Acquired immune deficiency syndrome. AIDS: the widening gyre. Nature. 1983;303:575-6.

[3] Barre-Sinoussi F, Chermann J, Rey F, Nugeyre M, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science. 1983;220:868-71.

[4] Gallo R, Sarin P, Gelmann E, Robert-Guroff M, Richardson E, Kalyanaraman V, et al. Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). Science. 1983;220:865-7.

[5] Coffin J, Haase A, Levy J, Montagnier L, Oroszlan S, Teich N, et al. What to call the AIDS virus? Nature. 1986;321 10.

[6] Clavel F, Guetard D, Brun-Vezinet F, Chamaret S, Rey M, Santos-Ferreira M, et al. Isolation of a new human retrovirus from West African patients with AIDS. Science. 1986;233:343-6.

[7] Daniel M, Letvin N, King N, Kannagi M, Sehgal P, Hunt R, et al. Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. Science. 1985;228:1201-4.

[8] Haase A. Pathogenesis of lentivirus infections. Nature. 1986;322 130-6.

[9] Mittler J, Markowitz M, Ho D, Perelson A. Improved estimates for HIV-1 clearance rate and intracellular delay. AIDS. 1999;13:1415-7.

[10] Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD. HIV-1 Dynamics in Vivo: Virion Clearance Rate, Infected Cell Life-Span, and Viral Generation Time. Science. 1996;271:1582-6.

[11] Ramratnam B, Bonhoeffer S, Binley J, Hurley A, Zhang L, Mittler JE, et al. Rapid production and clearance of HIV-1 and hepatitis C virus assessed by large volume plasma apheresis. The Lancet. 1999;354:1782-5.

[12] Ho D, Neumann A, Perelson A, Chen W, Leonard J, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature. 1995;373:123-6.

[13] Wei X, Ghosh S, Taylor M, Johnson V, Emini E, Deutsch P, et al. Viral dynamics in human immunodeficiency virus type 1 infection. Nature. 1995;373:117-22.

[14] Hahn BH, Shaw GM, De KM, Cock, Sharp PM. AIDS as a Zoonosis: Scientific and Public Health Implications. Science. 2000;287:607-14.

[15] Robertson DL, Anderson JP, Bradac JA, Carr JK, Foley B, Funkhouser RK, et al. HIV-1 Nomenclature Proposal. Science. 2000;288:55d-.

[16] De Leys R, Vanderborght B, Vanden Haesevelde M, Heyndrickx L, van Geel A, Wauters C, et al. Isolation and partial characterization of an unusual human immunodeficiency retrovirus from two persons of west-central African origin. J Virol. 1990;64:1207-16.

[17] Charneau P, Borman AM, Quillent C, Guétard D, Chamaret S, Cohen J, et al. Isolation and Envelope Sequence of a Highly Divergent HIV-1 Isolate: Definition of a New HIV-1 Group. Virology. 1994;205:247-53.

[18] Gurtler LG, Hauser PH, Eberle J, von Brunn A, Knapp S, Zekeng L, et al. A new subtype of human immunodeficiency virus type 1 (MVP-5180) from Cameroon. J Virol. 1994;68:1581-5.

[19] Vanden Haesevelde M, Decourt JL, De Leys RJ, Vanderborght B, van der Groen G, van Heuverswijn H, et al. Genomic cloning and complete sequence analysis of a highly divergent African human immunodeficiency virus isolate. J Virol. 1994;68:1586-96.

[20] Simon F, Mauclere P, Roques P, Loussert-Ajaka I, Muller-Trutwin MC, Saragosti S, et al. Identification of a new human immunodeficiency virus type 1 distinct from group M and group O. Nat Med. 1998;4:1032-7.

[21] Preston B, Poiesz B, Loeb L. Fidelity of HIV-1 reverse transcriptase. Science. 1988;242:1168-71.

[22] Jetzt AE, Yu H, Klarmann GJ, Ron Y, Preston BD, Dougherty JP. High Rate of Recombination throughout the Human Immunodeficiency Virus Type 1 Genome. J Virol. 2000;74:1234-40.

[23] Kanki P, McLane M, King N, Jr, Letvin N, Hunt R, Sehgal P, et al. Serologic identification and characterization of a macaque T-lymphotropic retrovirus closely related to HTLV-III. Science. 1985;228:1199-201.

[24] Letvin N, Daniel M, Sehgal P, Desrosiers R, Hunt R, Waldron L, et al. Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III. Science. 1985;230:71-3.

[25] Zhu T, Korber BT, Nahmias AJ, Hooper E, Sharp PM, Ho DD. An African HIV-1 sequence from 1959 and implications for the origin of the epidemic. Nature. 1998;391:594-7.

[26] Hillis DM. AIDS: Origins of HIV. Science. 2000;288:1757-9.

[27] Korber B, Muldoon M, Theiler J, Gao F, Gupta R, Lapedes A, et al. Timing the Ancestor of the HIV-1 Pandemic Strains. Science. 2000;288:1789-96.

[28] Alizon M, Sonigo P, Barré-Sinoussi F, Chermann J, Tiollais P, Montagnier L, et al. Molecular cloning of lymphadenopathy-associated virus. Nature. 1984;312:757-60.

[29] Luciw P, Potter S, Steimer K, Dina D, Levy J. Molecular cloning of AIDS-associated retrovirus. Nature. 1984;312:760-3.

[30] Trono D. When accessories turn out to be essential. Nat Med. 1998;4:1368-9.

[31] Cullen BR. HIV-1 Auxiliary Proteins: Making Connections in a Dying Cell. Cell. 1998;93:685-92.

[32] McKeating JA, McKnight A, Moore JP. Differential loss of envelope glycoprotein gp120 from virions of human immunodeficiency virus type 1 isolates: effects on infectivity and neutralization. J Virol. 1991;65:852-60.

[33] Pandori M, Fitch N, Craig H, Richman D, Spina C, Guatelli J. Producer-cell modification of human immunodeficiency virus type 1: Nef is a virion protein. J Virol. 1996;70:4283-90.

[34] Lu YL, Spearman P, Ratner L. Human immunodeficiency virus type 1 viral protein R localization in infected cells and virions. J Virol. 1993;67:6542-50.

[35] Liu H, Wu X, Newman M, Shaw G, Hahn B, Kappes J. The Vif protein of human and simian immunodeficiency viruses is packaged into virions and associates with viral core structures. J Virol. 1995;69:7630-8.

[36] Ott D, Coren L, Kane B, Busch L, Johnson D, Sowder R, 2nd, et al. Cytoskeletal proteins inside human immunodeficiency virus type 1 virions. J Virol. 1996;70:7734-43.

[37] Arthur L, Bess J, Jr, Sowder R, 2nd, Benveniste R, Mann D, Chermann J, et al. Cellular proteins bound to immunodeficiency viruses: implications for pathogenesis and vaccines. Science. 1992;258:1935-8.

[38] Franke E, Yuan H, Luban J. Specific incorporation of cyclophilin A into HIV-1 virions. Nature. 1994;372:359-62.

[39] Thali M, Bukovsky A, Kondo E, Rosenwirth B, Walsh C, Sodroski J, et al. Functional association of cyclophilin A with HIV-1 virions. Nature. 1994;372:363-5.

[40] McClure M, Sattentau Q, Beverley P, Hearn J, Fitzgerald A, Zuckerman A, et al. HIV infection of primate lymphocytes and conservation of the CD4 receptor. Nature. 1987;330:487-9.

[41] Stein BS, Gowda SD, Lifson JD, Penhallow RC, Bensch KG, Engleman EG. pH-independent HIV entry into CD4-positive T cells via virus envelope fusion to the plasma membrane. Cell. 1987;49:659-68.

[42] Stevenson M. Portals of entry:uncovering HIV nuclear transport pathways. Trends in Cell Biol. 1996;6:9-15.

[43] Bukrinskaya A, Brichacek B, Mann A, Stevenson M. Establishment of a functional human immunodeficiency virus type 1 (HIV-1) reverse transcription complex involves the cytoskeleton. J Exp Med. 1998;188:2113-25.

[44] Charneau P, Mirambeau G, Roux P, Paulous S, Buc H, Clavel F. HIV-1 Reverse Transcription A Termination Step at the Center of the Genome. J Mol Biol. 1994;241:651-62.

[45] Zennou V, Petit C, Guetard D, Nerhbass U, Montagnier L, Charneau P. HIV-1 Genome Nuclear Import Is Mediated by a Central DNA Flap. Cell. 2000;101:173-85.

[46] Bukrinsky M, Haggerty S, Dempsey M, Sharova N, Adzhubel A, Spitz L, et al. A nuclear localization signal within HIV-1 matrix protein that governs infection of non-dividing cells. Nature. 1993;365:666-9.

[47] Gallay P, Hope T, Chin D, Trono D. HIV-1 infection of nondividing cells through the recognition of integrase by the importin/karyopherin pathway. PNAS. 1997;94:9825-30.

[48] Popov S, Rexach M, Zybarth G, Reiling N, Lee M-A, Ratner L, et al. Viral protein R regulates nuclear import of the HIV-1 pre-integration complex. EMBO J. 1998;17:909-17.

[49] Cullen B. Regulation of HIV gene expression. AIDS. 1995;9:S19-32.

[50] Sakalian M, Hunter E. Molecular events in the assembly of retrovirus particles. Adv Exp Med Biol. 1998;440:329-39.

[51] Lifson J, Feinberg M, Reyes G, Rabin L, Banapour B, Chakrabarti S, et al. Induction of CD4-dependent cell fusion by the HTLV-III/LAV envelope glycoprotein. Nature. 1986;323:725-8.

[52] Lifson J, Reyes G, McGrath M, Stein B, Engleman E. AIDS retrovirus induced cytopathology: giant cell formation and involvement of CD4 antigen. Science. 1986;232:1123-7.

[53] Egger M, May M, Chene G, Phillips AN, Ledergerber B, Dabis F, et al. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. Lancet. 2002;360:119-29.

[54] Hogg RS, Yip B, Chan KJ, Wood E, Craib KJ, O'Shaughnessy MV, et al. Rates of disease progression by baseline CD4 cell count and viral load after initiating triple-drug therapy. JAMA. 2001;286:2568-77.

[55] Palella FJ, Jr., Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med. 1998;338:853-60.

[56] Porter K, Babiker A, Bhaskaran K, Darbyshire J, Pezzotti P, Walker AS. Determinants of survival following HIV-1 seroconversion after the introduction of HAART. Lancet. 2003;362:1267-74.

[57] Parker WB, White EL, Shaddix SC, Ross LJ, Buckheit RW, Jr., Germany JM, et al. Mechanism of inhibition of human immunodeficiency virus type 1 reverse transcriptase and human DNA polymerases alpha, beta, and gamma by the 5'-triphosphates of carbovir, 3'-azido-3'-deoxythymidine, 2',3'-dideoxyguanosine and 3'-deoxythymidine. A novel RNA template for the evaluation of antiretroviral drugs. J Biol Chem. 1991;266:1754-62.

[58] Esnouf R, Ren J, Ross C, Jones Y, Stammers D, Stuart D. Mechanism of inhibition of HIV-1 reverse transcriptase by non-nucleoside inhibitors. Nat Struct Biol. 1995;2:303-8.

[59] Spence RA, Kati WM, Anderson KS, Johnson KA. Mechanism of inhibition of HIV-1 reverse transcriptase by nonnucleoside inhibitors. Science. 1995;267:988-93.

[60] Flexner C. HIV-protease inhibitors. N Engl J Med. 1998;338:1281-92.

[61] Patick AK, Potts KE. Protease inhibitors as antiviral agents. Clin Microbiol Rev. 1998;11:614-27.

[62] Al-Mawsawi LQ, Al-Safi RI, Neamati N. Anti-infectives: clinical progress of HIV-1 integrase inhibitors. Expert Opin Emerg Drugs. 2008;13:213-25.

[63] DeJesus E, Berger D, Markowitz M, Cohen C, Hawkins T, Ruane P, et al. Antiviral activity, pharmacokinetics, and dose response of the HIV-1 integrase inhibitor GS-9137 (JTK-303) in treatment-naive and treatment-experienced patients. J Acquir Immune Defic Syndr. 2006;43:1-5.

[64] Hazuda D, Iwamoto M, Wenning L. Emerging pharmacology: inhibitors of human immunodeficiency virus integration. Annu Rev Pharmacol Toxicol. 2009;49:377-94.

[65] Hazuda DJ, Felock P, Witmer M, Wolfe A, Stillmock K, Grobler JA, et al. Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. Science. 2000;287:646-50.

[66] LaBranche CC, Galasso G, Moore JP, Bolognesi DP, Hirsch MS, Hammer SM. HIV fusion and its inhibition. Antivir Res. 2001;50:95-115.

[67] Lalezari JP, Henry K, O'Hearn M, Montaner JS, Piliero PJ, Trottier B, et al. Enfuvirtide, an HIV-1 fusion inhibitor, for drug-resistant HIV infection in North and South America. N Engl J Med. 2003;348:2175-85.

[68] Stebbing J, Bower M, Mandalia S, Nelson M, Gazzard B. Highly active anti-retroviral therapy (HAART)-induced maintenance of adaptive but not innate immune parameters is associated with protection from HIV-induced mortality. Clin Exp Immunol. 2006;145:271-6.

[69] Telenti A, Paolo Rizzardi G. Limits to potent antiretroviral therapy. Rev Med Virol. 2000;10:385-93.

[70] Sekaly RP. The failed HIV Merck vaccine study: a step back or a launching point for future vaccine development? J Exp Med. 2008;205:7-12.

[71] Leonard WJ. Cytokines and immunodeficiency diseases. Nat Rev Immunol. 2001;1:200-8.

[72] Leonard WJ, Spolski R. Interleukin-21: a modulator of lymphoid proliferation, apoptosis and differentiation. Nat Rev Immunol. 2005;5:688-98.

[73] Parrish-Novak J, Dillon S, Nelson A, Hammond A, Sprecher C, Gross J, et al. Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. Nature. 2000;408:57 - 63.

[74] Bondensgaard K, Breinholt J, Madsen D, Omkvist DH, Kang L, Worsaae A, et al. The existence of multiple conformers of interleukin-21 directs engineering of a superpotent analogue. J Biol Chem. 2007;282:23326-36.

[75] Rahman M, Nara H, Onoda T, Araki A, Li J, Hoshino T, et al. Cloning and characterization of an isoform of interleukin-21. FEBS Letters. 2007;581:4001-9.

[76] Kang L, Bondensgaard K, Li T, Hartmann R, Hjorth SA. Rational design of interleukin-21 antagonist through selective elimination of the gammaC binding epitope. J Biol Chem. 2010;285:12223-31.

[77] Coquet JM, Kyparissoudis K, Pellicci DG, Besra G, Berzins SP, Smyth MJ, et al. IL-21 is produced by NKT cells and modulates NKT cell activation and cytokine production. J Immunol. 2007;178:2827-34.

[78] Kim HP, Korn LL, Gamero AM, Leonard WJ. Calcium-dependent activation of interleukin-21 gene expression in T cells. J Biol Chem. 2005;280:25291-7.

[79] Mehta DS, Wurster AL, Weinmann AS, Grusby MJ. NFATc2 and T-bet contribute to T-helper-cell-subset-specific regulation of IL-21 expression. PNAS. 2005;102:2016-21.

[80] Bauquet A, Jin H, Paterson A, Mitsdoerffer M, Ho I, Sharpe A, et al. The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and TH-17 cells. Nat Immunol. 2009;10:167-75.

[81] Hiramatsu Y, Suto A, Kashiwakuma D, Kanari H, Kagami S-i, Ikeda K, et al. c-Maf activates the promoter and enhancer of the IL-21 gene, and TGF-{beta} inhibits c-Maf-induced IL-21 production in CD4+ T cells. J Leukoc Biol. 2010;87:703-12.

[82] Pot C, Jin H, Awasthi A, Liu SM, Lai C-Y, Madan R, et al. Cutting Edge: IL-27 Induces the Transcription Factor c-Maf, Cytokine IL-21, and the Costimulatory Receptor ICOS that Coordinately Act Together to Promote Differentiation of IL-10-Producing Tr1 Cells. J Immunol. 2009;183:797-801.
[83] Caprioli F, Sarra M, Caruso R, Stolfi C, Fina D, Sica G, et al. Autocrine Regulation of IL-21 Production in Human T Lymphocytes. J Immunol. 2008;180:1800-7.
[84] Suto A, Kashiwakuma D, Kagami S-i, Hirose K, Watanabe N, Yokote K, et al. Development and characterization of IL-21-producing CD4+ T cells. J Exp Med. 2008;205:1369-79.
[85] Harada M, Magara-Koyanagi K, Watarai H, Nagata Y, Ishii Y, Kojo S, et al. IL-21-induced B{varepsilon} cell apoptosis mediated by natural killer T cells suppresses IgE responses. J Exp Med. 2006;203:2929-37.
[86] Holm CK, Petersen CC, Hvid M, Petersen L, Paludan SR, Deleuran B, et al. TLR3 Ligand Polyinosinic:Polycytidylic Acid Induces IL-17A and IL-21 Synthesis in Human Th Cells. J Immunol. 2009;183:4422-31.
[87] Ozaki K, Kikly K, Michalovich D, Young P, Leonard W. Cloning of a type I cytokine receptor most related to the IL-2 receptor beta chain. PNAS. 2000;97:11439-44.
[88] Parrish-Novak J, Dillon SR, Nelson A, Hammond A, Sprecher C, Gross JA, et al. Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. Nature. 2000;408:57-63.
[89] Asao H, Okuyama C, Kumaki S, Ishii N, Tsuchiya S, Foster D, et al. Cutting Edge: The common {{gamma}}-chain is an indispensable subunit of the IL-21 receptor complex. J Immunol. 2001;167:1-5.
[90] Habib T, Senadheera S, Weinberg K, Kaushansky K. The common gamma chain (gamma c) is a required signaling component of the IL-21 receptor and supports IL-21-induced cell proliferation via JAK3. Biochemistry. 2002;41:8725-31.
[91] Zeng R, Spolski R, Casas E, Zhu W, Levy DE, Leonard WJ. The molecular basis of IL-21-mediated proliferation. Blood. 2007;109:4135-42.

[92] Asao H, Okuyama C, Kumaki S, Ishii N, Tsuchiya S, Foster D, et al. Cutting edge: the common gamma-chain is an indispensable subunit of the IL-21 receptor complex. J Immunol. 2001;167:1-5.

[93] Konforte D, Paige CJ. Identification of cellular intermediates and molecular pathways induced by IL-21 in human B cells. J Immunol. 2006;177:8381-92.

[94] Kwon H, Thierry-Mieg D, Thierry-Mieg J, Kim H-P, Oh J, Tunyaplin C, et al. Analysis of Interleukin-21-Induced Prdm1 Gene Regulation Reveals Functional Cooperation of STAT3 and IRF4 Transcription Factors. Immunity. 2009;31:941-52.

[95] Mizuguchi M, Asao H, Hara T, Higuchi M, Fujii M, Nakamura M. Transcriptional Activation of the Interleukin-21 Gene and Its Receptor Gene by Human T-cell Leukemia Virus Type 1 Tax in Human T-cells. J Biol Chem. 2009;284:25501-11.

[96] Nara H, Onoda T, Rahman M, Araki A, Juliana FM, Tanaka N, et al. Regulation of interleukin-21 receptor expression and its signal transduction by WSB-2. Biochem and Biophys Res Commun. 2010;392:171-7.

[97] Brandt K, Bulfone-Paus S, Foster DC, Ruckert R. Interleukin-21 inhibits dendritic cell activation and maturation. Blood. 2003;102:4090-8.

[98] Caruso R, Fina D, Peluso I, Fantini MC, Tosti C, Blanco GDV, et al. IL-21 is highly produced in Helicobacter pylori-infected gastric mucosa and promotes gelatinases synthesis. J Immunol. 2007;178:5957-65.

[99] Distler JH, Jungel A, Kowal-Bielecka O, Michel BA, Gay RE, Sprott H, et al. Expression of interleukin-21 receptor in epidermis from patients with systemic sclerosis. Arthritis Rheum. 2005;52:856-64.

[100] Jin H, Carrio R, Yu A, Malek TR. Distinct activation signals determine whether IL-21 induces B cell costimulation, growth arrest, or Bim-dependent apoptosis. J Immunol. 2004;173:657-65.

[101] Parrish-Novak J, Foster DC, Holly RD, Clegg CH. Interleukin-21 and the IL-21 receptor: novel effectors of NK and T cell responses. J Leukoc Biol. 2002;72:856-63.

[102] Ozaki K, Hishiya A, Hatanaka K, Nakajima H, Wang G, Hwu P, et al. Overexpression of interleukin 21 induces expansion of hematopoietic progenitor cells. Int J Hematol. 2006;84:224-30.

[103] Ozaki K, Spolski R, Feng CG, Qi C-F, Cheng J, Sher A, et al. A critical role for IL-21 in regulating immunoglobulin production. Science. 2002;298:1630-4.

[104] Jin H, Malek TR. Redundant and unique regulation of activated mouse B lymphocytes by IL-4 and IL-21. J Leukoc Biol. 2006;80:1416-23.

[105] Good KL, Bryant VL, Tangye SG. Kinetics of human B cell behavior and amplification of proliferative responses following stimulation with IL-21. J Immunol. 2006;177:5236-47.

[106] Wu Z, Kim H-P, Xue H-H, Liu H, Zhao K, Leonard WJ. Interleukin-21 receptor gene induction in human T cells is mediated by T-cell receptor-induced Sp1 activity. Mol Cell Biol. 2005;25:9741-52.

[107] Suto A, Nakajima H, Hirose K, Suzuki K, Kagami S, Seto Y, et al. Interleukin 21 prevents antigen-induced IgE production by inhibiting germ line C(epsilon) transcription of IL-4-stimulated B cells. Blood. 2002;100:4565-73.

[108] Wood N, Bourque K, Donaldson DD, Collins M, Vercelli D, Goldman SJ, et al. IL-21 effects on human IgE production in response to IL-4 or IL-13. Cell Immunol. 2004;231:133-45.

[109] Avery DT, Ma CS, Bryant VL, Santner-Nanan B, Nanan R, Wong M, et al. STAT3 is required for IL-21-induced secretion of IgE from human naive B cells. Blood. 2008;112:1784-93.

[110] Avery DT, Deenick EK, Ma CS, Suryani S, Simpson N, Chew GY, et al. B cell–intrinsic signaling through IL-21 receptor and STAT3 is required for establishing long-lived antibody responses in humans. J Exp Med. 2010;207:155-71.

[111] Kobayashi S, Haruo N, Sugane K, Ochs HD, Agematsu K. Interleukin-21 stimulates B-cell immunoglobulin E synthesis in human beings concomitantly with activation-induced cytidine deaminase expression and differentiation into plasma cells. Hum Immunol. 2009;70:35-40.

[112] Mehta DS, Wurster AL, Whitters MJ, Young DA, Collins M, Grusby MJ. IL-21 induces the apoptosis of resting and activated primary B cells. J Immunol. 2003;170:4111-8.

[113] Ozaki K, Spolski R, Ettinger R, Kim H-P, Wang G, Qi C-F, et al. Regulation of B cell differentiation and plasma cell generation by IL-21, a novel Inducer of Blimp-1 and Bcl-6. J Immunol. 2004;173:5361-71.

[114] Calame KL, Lin KI, Tunyaplin C. Regulatory mechanisms that determine the development and function of plasma cells. Annu Rev Immunol. 2003;21:205-30.

[115] Shaffer AL, Lin KI, Kuo TC, Yu X, Hurt EM, Rosenwald A, et al. Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program. Immunity. 2002;17:51-62.

[116] Ettinger R, Sims GP, Fairhurst A-M, Robbins R, da Silva YS, Spolski R, et al. IL-21 induces differentiation of human naive and memory B cells into antibody-secreting plasma cells. J Immunol. 2005;175:7867-79.

[117] Pene J, Gauchat JF, Lecart S, Drouet E, Guglielmi P, Boulay V, et al. Cutting edge: IL-21 is a switch factor for the production of IgG1 and IgG3 by human B cells. J Immunol. 2004;172:5154-7.

[118] Avery DT, Bryant VL, Ma CS, de Waal Malefyt R, Tangye SG. IL-21-Induced Isotype Switching to IgG and IgA by Human Naive B Cells Is Differentially Regulated by IL-4. J Immunol. 2008;181:1767-79.

[119] Borte S, Pan-Hammarstrom Q, Liu C, Sack U, Borte M, Wagner U, et al. Interleukin-21 restores immunoglobulin production ex vivo in patients with common variable immunodeficiency and selective IgA deficiency. Blood. 2009;114:4089-98.

[120] Ettinger R, Sims GP, Robbins R, Withers D, Fischer RT, Grammer AC, et al. IL-21 and BAFF/BLyS synergize in stimulating plasma cell differentiation from a unique population of human splenic memory B cells. J Immunol. 2007;178:2872-82.

[121] Ng LG, Mackay CR, Mackay F. The BAFF/APRIL system: life beyond B lymphocytes. Mol Immunol. 2005;42:763-72.

[122] Dienz O, Eaton SM, Bond JP, Neveu W, Moquin D, Noubade R, et al. The induction of antibody production by IL-6 is indirectly mediated by IL-21 produced by CD4+ T cells. J Exp Med. 2009;206:69-78.

[123] Yoon S-O, Zhang X, Berner P, Choi YS. IL-21 and IL-10 have redundant roles but differential capacities at different stages of plasma cell generation from human germinal center B cells. J Leukoc Biol. 2009;86:1311-8.

[124] Linterman MA, Beaton L, Yu D, Ramiscal RR, Srivastava M, Hogan JJ, et al. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. J Exp Med. 2010;207:353-63.

[125] Bessa J, Kopf M, Bachmann MF. Cutting edge: IL-21 and TLR signaling regulate germinal center responses in a B cell-intrinsic manner. J Immunol. 2010;184:4615-9.

[126] Zotos D, Coquet JM, Zhang Y, Light A, D'Costa K, Kallies A, et al. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell–intrinsic mechanism. J Exp Med. 2010;207:365-78.

[127] Wurster AL, Rodgers VL, Satoskar AR, Whitters MJ, Young DA, Collins M, et al. Interleukin 21 is a T helper (Th) cell 2 cytokine that specifically inhibits the differentiation of naive Th cells into Interferon {gamma}-producing Th1 cells. J Exp Med. 2002;196:969-77.

[128] Chtanova T, Tangye SG, Newton R, Frank N, Hodge MR, Rolph MS, et al. T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells. J Immunol. 2004;173:68-78.

[129] Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, et al. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. Nature. 2007;448:480-3.

[130] Korn T, Bettelli E, Gao W, Awasthi A, Jager A, Strom TB, et al. IL-21 initiates an alternative pathway to induce proinflammatory TH17 cells. Nature. 2007;448:484-7.

[131] Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, et al. IL-6 programs TH-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat Immunol. 2007;8:967-74.

[132] Suto A, Wurster AL, Reiner SL, Grusby MJ. IL-21 inhibits IFN-{gamma} production in developing Th1 cells through the repression of eomesodermin expression. J Immunol. 2006;177:3721-7.

[133] Strengell M, Sareneva T, Foster D, Julkunen I, Matikainen S. IL-21 up-regulates the expression of genes associated with innate immunity and Th1 response. J Immunol. 2002;169:3600-5.

[134] Pesce J, Kaviratne M, Ramalingam TR, Thompson RW, Urban JF, Cheever AW, et al. The IL-21 receptor augments Th2 effector function and alternative macrophage activation. J Clin Invest. 2006;116:2044-55.

[135] Frohlich A, Marsland BJ, Sonderegger I, Kurrer M, Hodge MR, Harris NL, et al. IL-21 receptor signaling is integral to the development of Th2 effector responses in vivo. Blood. 2007;109:2023-31.

[136] Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol. 2005;6:1123-32.

[137] Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol. 2005;6:1133-41.

[138] Yang L, Anderson DE, Baecher-Allan C, Hastings WD, Bettelli E, Oukka M, et al. IL-21 and TGF-[bgr] are required for differentiation of human TH17 cells. Nature. 2008;454:350-2.

[139] Li MO, Sanjabi S, Flavell RA. Transforming growth factor-beta controls development, homeostasis, and tolerance of T cells by regulatory T cell-dependent and -independent mechanisms. Immunity. 2006;25:455-71.

[140] Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, et al. Transforming growth factor-beta induces development of the T(H)17 lineage. Nature. 2006;441:231-4.

[141] Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006;441:235-8.

[142] Kastelein RA, Hunter CA, Cua DJ. Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. Annu Rev Immunol. 2007;25:221-42.

[143] Bettelli E, Oukka M, Kuchroo VK. T(H)-17 cells in the circle of immunity and autoimmunity. Nat Immunol. 2007;8:345-50.

[144] Ivanov, II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell. 2006;126:1121-33.

[145] Huber M, Brüstle A, Reinhard K, Guralnik A, Walter G, Mahiny A, et al. IRF4 is essential for IL-21-mediated induction, amplification, and stabilization of the Th17 phenotype. PNAS. 2008;105:20846-51.

[146] Chen Q, Yang W, Gupta S, Biswas P, Smith P, Bhagat G, et al. IRF-4-Binding Protein Inhibits Interleukin-17 and Interleukin-21 Production by Controlling the Activity of IRF-4 Transcription Factor. Immunity. 2008;29:899-911.

[147] Peluso I, Fantini MC, Fina D, Caruso R, Boirivant M, MacDonald TT, et al. IL-21 counteracts the regulatory T cell-mediated suppression of human CD4+ T lymphocytes. J Immunol. 2007;178:732-9.

[148] Li Y, Yee C. IL-21 mediated Foxp3 suppression leads to enhanced generation of antigen-specific CD8+ cytotoxic T lymphocytes. Blood. 2008;111:229 - 35.

[149] Zeng R, Spolski R, Finkelstein S, Oh S, Kovanen P, Hinrichs C, et al. Synergy of IL-21 and IL-15 in regulating CD8 T cell expansion and function. J Exp Med. 2005;201:130-48.

[150] Schluns KS, Kieper WC, Jameson SC, Lefrancois L. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells in vivo. Nat Immunol. 2000;1:426-32.

[151] Zhang X, Sun S, Hwang I, Tough DF, Sprent J. Potent and selective stimulation of memory-phenotype CD8+ T cells in vivo by IL-15. Immunity. 1998;8:591-9.

[152] Hogg AE, Bowick GC, Herzog NK, Cloyd MW, Endsley JJ. Induction of granulysin in CD8+ T cells by IL-21 and IL-15 is suppressed by human immunodeficiency virus-1. J Leukoc Biol. 2009;86:1191-203.

[153] Alves NL, Arosa FA, van Lier RA. IL-21 sustains CD28 expression on IL-15-activated human naive CD8+ T cells. J Immunol. 2005;175:755-62.

[154] Li Y, Bleakley M, Yee C. IL-21 influences the frequency, phenotype, and affinity of the antigen-specific CD8 T cell response. J Immunol. 2005;175:2261-9.

[155] White L, Krishnan S, Strbo N, Liu H, Kolber MA, Lichtenheld MG, et al. Differential effects of IL-21 and IL-15 on perforin expression, lysosomal degranulation,

and proliferation in CD8 T cells of patients with human immunodeficiency virus-1 (HIV). Blood. 2007;109:3873-80.

[156] Nguyen H, Weng N-p. IL-21 preferentially enhances IL-15-mediated homeostatic proliferation of human CD28+ CD8 memory T cells throughout the adult age span. J Leukoc Biol. 2010;87:43-9.

[157] Spolski R, Leonard WJ. IL-21 and T follicular helper cells. Int Immunol. 2010;22:7-12.

[158] Linterman M, Vinuesa C. Signals that influence T follicular helper cell differentiation and function. Semin in Immunopathol. 2010.

[159] Nurieva RI, Chung Y, Hwang D, Yang XO, Kang HS, Ma L, et al. Generation of T Follicular Helper Cells Is Mediated by Interleukin-21 but Independent of T Helper 1, 2, or 17 Cell Lineages. Immunity. 2008;29:138-49.

[160] Vogelzang A, McGuire HM, Yu D, Sprent J, Mackay CR, King C. A Fundamental Role for Interleukin-21 in the Generation of T Follicular Helper Cells. Immunity. 2008;29:127-37.

[161] Schmitt N, Morita R, Bourdery L, Bentebibel SE, Zurawski SM, Banchereau J, et al. Human Dendritic Cells Induce the Differentiation of Interleukin-21-Producing T Follicular Helper-like Cells through Interleukin-12. Immunity. 2009;31:158-69.

[162] Spolski R, Leonard WJ. The Yin and Yang of interleukin-21 in allergy, autoimmunity and cancer. Curr Opin Immunol. 2008;20:295-301.

[163] Cao X, Shorese E, Hu-Lib J, Anverg M, Kelsailc B, Russella S, et al. Defective lymphoid development in mice lacking expression of the common cytokine receptor γ chain. Immunity. 1995;2:223-38.

[164] Di Santo JP, Vosshenrich CAJ. Bone marrow versus thymic pathways of natural killer cell development. Immunol Rev. 2006;214:35-46.

[165] Kasaian MT, Whitters MJ, Carter LL, Lowe LD, Jussif JM, Deng B, et al. IL-21 limits NK cell responses and promotes antigen-specific T cell activation: a mediator of the transition from innate to adaptive immunity. Immunity. 2002;16:559-69.

[166] Vosshenrich CAJ, Ranson T, Samson SI, Corcuff E, Colucci F, Rosmaraki EE, et al. Roles for common cytokine receptor {gamma}-chain-dependent cytokines in the

generation, differentiation, and maturation of NK cell precursors and peripheral NK cells in vivo. J Immunol. 2005;174:1213-21.

[167] Sivori S, Cantoni C, Parolini S, Marcenaro E, Conte R, Moretta L, et al. IL-21 induces both rapid maturation of human CD34+ cell precursors towards NK cells and acquisition of surface killer Ig-like receptors. Eur J Immunol. 2003;33:3439 - 47.

[168] Bonanno G, Mariotti A, Procoli A, Corallo M, Scambia G, Pierelli L, et al. Interleukin-21 induces the differentiation of human umbilical cord blood CD34-lineage- cells into pseudomature lytic NK cells. BMC Immunol. 2009;10:46.

[169] Toomey J, Gays F, Foster D, Brooks C. Cytokine requirements for the growth and development of mouse NK cells in vitro. J Leukoc Biol. 2003;74:233 - 42.

[170] Brady J, Hayakawa Y, Smyth M, Nutt S. IL-21 induces the functional maturation of murine NK cells. J Immunol. 2004;172:2048-58.

[171] Gays F, Martin K, Kenefeck R, Aust JG, Brooks CG. Multiple cytokines regulate the NK gene complex-encoded receptor repertoire of mature NK cells and T cells. J Immunol. 2005;175:2938-47.

[172] Burgess SJ, Marusina AI, Pathmanathan I, Borrego F, Coligan JE. IL-21 down-regulates NKG2D/DAP10 expression on human NK and CD8+ T cells. J Immunol. 2006;176:1490-7.

[173] Wang G, Tschoi M, Spolski R, Lou Y, Ozaki K, Feng C, et al. In vivo antitumor activity of interleukin 21 mediated by Natural Killer cells. Cancer Res. 2003;63:9016-22.

[174] Godfrey DI, Kronenberg M. Going both ways: immune regulation via CD1d-dependent NKT cells. J Clin Invest. 2004;114:1379-88.

[175] Pelletier M, Bouchard A, Girard D. In vivo and in vitro roles of IL-21 in inflammation. J Immunol. 2004;173:7521-30.

[176] Rückert R, Bulfone-Paus S, Brandt K. Interleukin-21 stimulates antigen uptake, protease activity, survival and induction of CD4<sup>+</sup> T cell proliferation by murine macrophages. Clin & Exp Immunol. 2008;151:487-95.

[177] Bubier JA, Sproule TJ, Foreman O, Spolski R, Shaffer DJ, Morse HC, et al. A critical role for IL-21 receptor signaling in the pathogenesis of systemic lupus erythematosus in BXSB-Yaa mice. PNAS. 2009;106:1518-23.

[178] Herber D, Brown TP, Liang S, Young DA, Collins M, Dunussi-Joannopoulos K. IL-21 has a pathogenic role in a Lupus-prone mouse model and its blockade with IL-21R.Fc reduces disease progression. J Immunol. 2007;178:3822-30.

[179] Young D, Hegen M, Ling H, Ma M, Whitters M, Albert L, et al. Blockade of the interleukin-21/interleukin-21 receptor pathway ameliorates disease in animal models of rheumatoid arthritis. Arthr & Rheum. 2007;56:1152-63.

[180] Webb R, Merrill JT, Kelly JA, Sestak A, Kaufman KM, Langefeld CD, et al. A polymorphism within IL21R confers risk for systemic lupus erythematosus. Arthr & Rheum. 2009;60:2402-7.

[181] Sawalha AH, Kaufman KM, Kelly JA, Adler AJ, Aberle T, Kilpatrick J, et al. Genetic association of IL-21 polymorphisms with systemic lupus erythematosus. Ann Rheum Dis. 2007:ard.2007.075424.

[182] Liu L, Xu Y, Wang J, Li H. Upregulated IL-21 and IL-21 receptor expression is involved in experimental autoimmune uveitis (EAU). Mol Vis. 2010;15:2938-44.

[183] Vinuesa CG, Cook MC, Angelucci C, Athanasopoulos V, Rui L, Hill KM, et al. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. Nature. 2005;435:452-8.

[184] King C, Ilic A, Koelsch K, Sarvetnick N. Homeostatic expansion of T cells during immune insufficiency generates autoimmunity. Cell. 2004;117:265-77.

[185] Yamanouchi J, Rainbow D, Serra P, Howlett S, Hunter K, Garner VE, et al. Interleukin-2 gene variation impairs regulatory T cell function and causes autoimmunity. Nat Genet. 2007;39:329-37.

[186] Spolski R, Kashyap M, Robinson C, Yu Z, Leonard WJ. IL-21 signaling is critical for the development of type I diabetes in the NOD mouse. PNAS. 2008;105:14028-33.

[187] Sutherland APR, Van Belle T, Wurster AL, Suto A, Michaud M, Zhang D, et al. Interleukin-21 Is Required for the Development of Type 1 Diabetes in NOD Mice. Diabetes. 2009;58:1144-55.

[188] McGuire HM, Vogelzang A, Hill N, Flodström-Tullberg M, Sprent J, King C. Loss of parity between IL-2 and IL-21 in the NOD Idd3 locus. PNAS. 2009;106:19438-43.

[189] De Nitto D, Monteleone I, Franzè E, Pallone F, Monteleone G. Involvement of interleukin-15 and interleukin-21, two gamma-chain-related cytokines, in celiac disease. World J Gastroenterol. 2009;15:4609-14.

[190] Jones JL, Phuah C-L, Cox AL, Thompson SA, Ban M, Shawcross J, et al. IL-21 drives secondary autoimmunity in patients with multiple sclerosis, following therapeutic lymphocyte depletion with alemtuzumab (Campath-1H). J Clin Invest. 2009;119:2052-61.

[191] Coquet JM, Chakravarti S, Smyth MJ, Godfrey DI. Cutting Edge: IL-21 Is Not Essential for Th17 Differentiation or Experimental Autoimmune Encephalomyelitis. J Immunol. 2008;180:7097-101.

[192] Sonderegger I, Kisielow J, Meier R, King C, Kopf M. IL-21 and IL-21R are not required for development of Th17 cells and autoimmunity <I>in vivo</I>. Eur J Immunol. 2008;38:1833-8.

[193] Vollmer TL, Liu R, Price M, Rhodes S, La Cava A, Shi FD. Differential effects of IL-21 during initiation and progression of autoimmunity against neuroantigen. J Immunol. 2005;174:2696-701.

[194] Piao WH, Jee YH, Liu RL, Coons SW, Kala M, Collins M, et al. IL-21 modulates CD4+ CD25+ regulatory T-cell homeostasis in experimental autoimmune encephalomyelitis. Scand J Immunol. 2008;67:37-46.

[195] Niu X, He D, Zhang X, Yue T, Li N, Zhang JZ, et al. IL-21 regulates Th17 cells in rheumatoid arthritis. Hum Immunol. 2010;71:334-41.

[196] Jang E, Cho S-H, Park H, Paik D-J, Kim JM, Youn J. A Positive Feedback Loop of IL-21 Signaling Provoked by Homeostatic CD4+CD25- T Cell Expansion Is Essential for the Development of Arthritis in Autoimmune K/BxN Mice. J Immunol. 2009;182:4649-56.

[197] Andersson AK, Feldmann M, Brennan FM. Neutralizing IL-21 and IL-15 Inhibits Pro-inflammatory Cytokine Production in Rheumatoid Arthritis. Scandin J Immunol. 2008;68:103-11.

[198] Yamamoto-Furusho JK, Miranda-Pérez E, Fonseca-Camarillo G, Sánchez-Muñoz F, Barreto-Zuñiga R, Dominguez-Lopez A. Interleukin 21 expression is increased in rectal biopsies from patients with ulcerative colitis. Inflamm Bowel Dis. 2009;9999:NA.

[199] Festen EAM, Goyette P, Scott R, Annese V, Zhernakova A, Lian J, et al. Genetic variants in the region harbouring IL2/IL21 associated with ulcerative colitis. Gut. 2009;58:799-804.

[200] Caruso R, Botti E, Sarra M, Esposito M, Stolfi C, Diluvio L, et al. Involvement of interleukin-21 in the epidermal hyperplasia of psoriasis. Nat Med. 2009;15:1013-5.

[201] Sarra M, Monteleone I, Stolfi C, Fantini MC, Sileri P, Sica G, et al. Interferon-gamma-expressing cells are a major source of interleukin-21 in inflammatory bowel diseases. Inflamm Bowel Dis. 2010.

[202] Hiromura Y, Kishida T, Nakano H, Hama T, Imanishi J, Hisa Y, et al. IL-21 administration into the nostril alleviates murine allergic rhinitis. J Immunol. 2007;179:7157-65.

[203] Kishida T, Hiromura Y, Shin-Ya M, Asada H, Kuriyama H, Sugai M, et al. IL-21 induces inhibitor of differentiation 2 and leads to complete abrogation of anaphylaxis in mice. J Immunol. 2007;179:8554-61.

[204] Jin H, Oyoshi MK, Le Y, Bianchi T, Koduru S, Mathias CB, et al. IL-21R is essential for epicutaneous sensitization and allergic skin inflammation in humans and mice. J Clin Invest. 2009;119:47-60.

[205] Frederiksen K, Lundsgaard D, Freeman J, Hughes S, Holm T, Skrumsager B, et al. IL-21 induces in vivo immune activation of NK cells and CD8+ T cells in patients with metastatic melanoma and renal cell carcinoma. Cancer Immunol Immunother. 2008;57:1439-49.

[206] Petersen CC, Diernaes JEF, Skovbo A, Hvid M, Deleuran B, Hokland M. Interleukin-21 restrains tumor growth and induces a substantial increase in the number of circulating tumor-specific T cells in a murine model of malignant melanoma. Cytokine. 2010;49:80-8.

[207] He H, Wisner P, Yang G, Hu H-M, Haley D, Miller W, et al. Combined IL-21 and low-Dose IL-2 therapy induces anti-tumor immunity and long-term curative effects in a murine melanoma tumor model. J Transl Med. 2006;4:24.

[208] Pouw N, Treffers-Westerlaken E, Kraan J, Wittink F, ten Hagen T, Verweij J, et al. Combination of IL-21 and IL-15 enhances tumour-specific cytotoxicity and cytokine

production of TCR-transduced primary T cells. Cancer Immunol Immunother. 2010;59:921-31.

[209] Markley JC, Sadelain M. Interleukin (IL)-7 and IL-21 are superior to IL-2 and IL-15 in promoting human T-cell-mediated rejection of systemic lymphoma in immunodeficient mice. Blood. 2010.

[210] Zhao F, Dou J, He XF, Wang J, Chu L, Hu W, et al. Enhancing therapy of B16F10 melanoma efficacy through tumor vaccine expressing GPI-anchored IL-21 and secreting GM-CSF in mouse model. Vaccine. 2010;28:2846-52.

[211] Williams P, Rafei M, Bouchentouf M, Raven J, Yuan S, Cuerquis J, et al. A Fusion of GMCSF and IL-21 Initiates Hypersignaling Through the IL-21Ralpha Chain With Immune Activating and Tumoricidal Effects In Vivo. Mol Ther. 2010.

[212] Kim-Schulze S, Kim HS, Fan Q, Kim DW, Kaufman HL. Local IL-21 Promotes the Therapeutic Activity of Effector T cells by Decreasing Regulatory T Cells Within the Tumor Microenvironment. Mol Ther. 2008;17:380-8.

[213] Skak K, Søndergaard H, Frederiksen KS, Ehrnrooth E. In vivo antitumor efficacy of interleukin-21 in combination with chemotherapeutics. Cytokine. 2009;48:231-8.

[214] Smyth MJ, Hayakawa Y, Cretney E, Zerafa N, Sivakumar P, Yagita H, et al. IL-21 enhances tumor-specific CTL induction by anti-DR5 antibody therapy. J Immunol. 2006;176:6347-55.

[215] Smyth MJ, Crowe NY, Pellicci DG, Kyparissoudis K, Kelly JM, Takeda K, et al. Sequential production of interferon-gamma by NK1.1(+) T cells and natural killer cells is essential for the antimetastatic effect of alpha-galactosylceramide. Blood. 2002;99:1259-66.

[216] Smyth MJ, Wallace ME, Nutt SL, Yagita H, Godfrey DI, Hayakawa Y. Sequential activation of NKT cells and NK cells provides effective innate immunotherapy of cancer. J Exp Med. 2005;201:1973-85.

[217] Davis I, Skrumsager B, Cebon J, Nicholaou T, Barlow J, Moller N, et al. An open-label, two-arm, phase I trial of recombinant human interleukin-21 in patients with metastatic melanoma. Clin Cancer Res. 2007;13:3630-6.

[218] Davis ID, Brady B, Kefford RF, Millward M, Cebon J, Skrumsager BK, et al. Clinical and Biological Efficacy of Recombinant Human Interleukin-21 in Patients with

Stage IV Malignant Melanoma without Prior Treatment: A Phase IIa Trial. Clin Cancer Res. 2009;15:2123-9.

[219] Watanabe M, Kono K, Kawaguchi Y, Mizukami Y, Mimura K, Maruyama T, et al. Interleukin-21 can efficiently restore impaired antibody-dependent cell-mediated cytotoxicity in patients with oesophageal squamous cell carcinoma. Br J Cancer. 2009;102:520-9.

[220] Castermans K, Tabruyn SP, Zeng R, van Beijnum JR, Eppolito C, Leonard WJ, et al. Angiostatic activity of the antitumor cytokine interleukin-21. Blood. 2008;112:4940-7.

[221] Sarosiek KA, Malumbres R, Nechushtan H, Gentles AJ, Avisar E, Lossos IS. Novel IL-21 signaling pathway up-regulates c-Myc and induces apoptosis of diffuse large B-cell lymphomas. Blood. 2010;115:570-80.

[222] Gelebart P, Zak Z, Anand M, Dien-Bard J, Amin HM, Lai R. Interleukin-21 effectively induces apoptosis in mantle cell lymphoma through a STAT1-dependent mechanism. Leukemia. 2009;23:1836-46.

[223] Sondergaard H, Coquet JM, Uldrich AP, McLaughlin N, Godfrey DI, Sivakumar PV, et al. Endogenous IL-21 Restricts CD8+ T Cell Expansion and Is not Required for Tumor Immunity. J Immunol. 2009;183:7326-36.

[224] Bucher C, Koch L, Vogtenhuber C, Goren E, Munger M, Panoskaltsis-Mortari A, et al. IL-21 blockade reduces graft-versus-host disease mortality by supporting inducible T regulatory cell generation. Blood. 2009;114:5375-84.

[225] Meguro A, Ozaki K, Oh I, Hatanaka K, Matsu H, Tatara R, et al. IL-21 is critical for GVHD in a mouse model. Bone Marrow Transplant. 2010;45:723-9.

[226] Thedrez A, Harly C, Morice A, Salot S, Bonneville M, Scotet E. IL-21-Mediated Potentiation of Antitumor Cytolytic and Proinflammatory Responses of Human V{gamma}9V{delta}2 T Cells for Adoptive Immunotherapy. J Immunol. 2009;182:3423-31.

[227] Battegay M, Moskophidis D, Rahemtulla A, Hengartner H, Mak TW, Zinkernagel RM. Enhanced establishment of a virus carrier state in adult CD4+ T-cell-deficient mice. J Virol. 1994;68:4700-4.

[228] Matloubian M, Concepcion RJ, Ahmed R. CD4+ T cells are required to sustain CD8+ cytotoxic T-cell responses during chronic viral infection. J Virol. 1994;68:8056-63.

[229] Ou R, Zhou S, Huang L, Moskophidis D. Critical role for alpha/beta and gamma interferons in persistence of lymphocytic choriomeningitis virus by clonal exhaustion of cytotoxic T cells. J Virol. 2001;75:8407-23.

[230] Elsaesser H, Sauer K, Brooks DG. IL-21 is required to control chronic viral infection. Science. 2009:1174182.

[231] Frohlich A, Kisielow J, Schmitz I, Freigang S, Shamshiev AT, Weber J, et al. IL-21R on T cells is critical for sustained functionality and control of chronic viral infection. Science. 2009:1172815.

[232] Yi JS, Du M, Zajac AJ. A vital role for interleukin-21 in the control of a chronic viral infection. Science. 2009:1175194.

[233] Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature. 2006;439:682-7.

[234] Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature. 2006;443:350-4.

[235] Brooks DG, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, Oldstone MB. Interleukin-10 determines viral clearance or persistence in vivo. Nat Med. 2006;12:1301-9.

[236] Ejrnaes M, Filippi CM, Martinic MM, Ling EM, Togher LM, Crotty S, et al. Resolution of a chronic viral infection after interleukin-10 receptor blockade. J Exp Med. 2006;203:2461-72.

[237] Brooks DG, Lee AM, Elsaesser H, McGavern DB, Oldstone MB. IL-10 blockade facilitates DNA vaccine-induced T cell responses and enhances clearance of persistent virus infection. J Exp Med. 2008;205:533-41.

[238] Brooks DG, Walsh KB, Elsaesser H, Oldstone MB. IL-10 directly suppresses CD4 but not CD8 T cell effector and memory responses following acute viral infection. Proc Natl Acad Sci U S A. 2010;107:3018-23.

[239] Saïdi H, Melki M-T, Gougeon M-L. HMGB1-Dependent Triggering of HIV-1 Replication and Persistence in Dendritic Cells as a Consequence of NK-DC Cross-Talk. PLoS ONE. 2008;3:e3601.

[240] Melki MT, Saidi H, Dufour A, Olivo-Marin JC, Gougeon ML. Escape of HIV-1-infected dendritic cells from TRAIL-mediated NK cell cytotoxicity during NK-DC cross-talk--a pivotal role of HMGB1. PLoS Pathog. 2010;6:e1000862.

[241] Reitano KN, Kottilil S, Gille CM, Zhang X, Yan M, O'Shea MA, et al. Defective Plasmacytoid Dendritic Cell-NK Cell Cross-Talk in HIV Infection. AIDS Res and Hum Retrov. 2009;25:1029-37.

[242] Conry SJ, Milkovich KA, Yonkers NL, Rodriguez B, Bernstein HB, Asaad R, et al. Impaired Plasmacytoid Dendritic Cell (PDC)-NK Cell Activity in Viremic Human Immunodeficiency Virus Infection Attributable to Impairments in both PDC and NK Cell Function. J Virol. 2009;83:11175-87.

[243] Hong HS, Bhatnagar N, Ballmaier M, Schubert U, Henklein P, Volgmann T, et al. Exogenous HIV-1 Vpr disrupts IFN-[alpha] response by plasmacytoid dendritic cells (pDCs) and subsequent pDC/NK interplay. Immunol Letters. 2009;125:100-4.

[244] Chehimi J, Papasavvas E, Tomescu C, Gekonge B, Abdulhaqq S, Raymond A, et al. Inability of plasmacytoid dendritic cells to directly lyse HIV-infected autologous CD4+ T cells despite induction of tumor necrosis factor-related apoptosis-inducing ligand. J Virol. 2010;84:2762-73.

[245] Scott-Algara D, Arnold V, Didier C, Kattan T, Pirozzi G, Barré-Sinoussi F, et al. The CD85j+ NK Cell Subset Potently Controls HIV-1 Replication in Autologous Dendritic Cells. PLoS ONE. 2008;3:e1975.

[246] Alter G, Kavanagh D, Rihn S, Luteijn R, Brooks D, Oldstone M, et al. IL-10 induces aberrant deletion of dendritic cells by natural killer cells in the context of HIV infection. J Clin Invest. 2010.

[247] Vieillard V, Strominger JL, Debre P. NK cytotoxicity against CD4+ T cells during HIV-1 infection: A gp41 peptide induces the expression of an NKp44 ligand. PNAS. 2005;102:10981-6.

[248] Vieillard V, Le Grand R, Dausset J, Debré P. A vaccine strategy against AIDS: An HIV gp41 peptide immunization prevents NKp44L expression and CD4+ T cell depletion in SHIV-infected macaques. PNAS. 2008;105:2100-4.

[249] Vieillard V, Habib R, Brochard P, Delache B, Bovendo H, Calvo J, et al. CCR5 or CXCR4 use influences the relationship between CD4 cell depletion, NKp44L expression and NK cytotoxicity in SHIV-infected macaques. AIDS. 2008;22:185-92.

[250] Fausther-Bovendo H, Sol-Foulon N, Candotti D, Agut H, Schwartz O, Debré P, et al. HIV escape from natural killer cytotoxicity: nef inhibits NKp44L expression on CD4+ T cells. AIDS. 2009;2:1077-87.

[251] Richard J, Sindhu S, Pham T, Belzile J, Cohen E. HIV-1 Vpr up-regulates expression of ligands for the activating NKG2D receptor and promotes NK cell-mediated killing. Blood. 2010;115:1354-63.

[252] Ward J, Davis Z, DeHart J, Zimmerman E, Bosque A, Brunetta E, et al. HIV-1 Vpr Triggers Natural Killer Cell–Mediated Lysis of Infected Cells through Activation of the ATR-Mediated DNA Damage Response. PLoS Pathog. 2009;5:e1000613.

[253] Liu Q, Sun Y, Rihn S, Nolting A, Tsoukas PN, Jost S, et al. Matrix metalloprotease inhibitors restore impaired NK cell-mediated antibody-dependent cellular cytotoxicity in human immunodeficiency virus type 1 infection. J Virol. 2009;83:8705-12.

[254] Brunetta E, Fogli M, Varchetta S, Bozzo L, Hudspeth KL, Marcenaro E, et al. Chronic HIV-1 viremia reverses NKG2A/NKG2C ratio on natural killer cells in patients with human cytomegalovirus co-infection. AIDS. 2010;24:27-34.

[255] Brunetta E, Fogli M, Varchetta S, Bozzo L, Hudspeth K, Marcenaro E, et al. The decreased expression of Siglec-7 represents an early marker of dysfunctional natural killer-cell subsets associated with high levels of HIV-1 viremia. Blood. 2009;114:3822-30.

[256] Leeansyah E, Zhou J, Paukovics G, Lewin SR, Crowe SM, Jaworowski A. Decreased NK Cell FcRgamma in HIV-1 infected individuals receiving combination antiretroviral therapy: a cross sectional study. PLoS ONE. 2010;5:e9643.

[257] Hong HS, Eberhard JM, Keudel P, Bollmann BA, Ballmaier M, Bhatnagar N, et al. HIV infection is associated with a preferential decline in less-differentiated CD56dim CD16+ NK cells. J Virol. 2010;84:1183-8.

[258] Reeves RK, Gillis J, Wong FE, Yu Y, Connole M, Johnson RP. CD16- natural killer cells: enrichment in mucosal and secondary lymphoid tissues and altered function during chronic SIV infection. Blood. 2010.

[259] Tiemessen CT, Shalekoff S, Meddows-Taylor S, Schramm DB, Papathanasopoulos MA, Gray GE, et al. Cutting Edge: Unusual NK cell responses to HIV-1 peptides are associated with protection against maternal-infant transmission of HIV-1. J Immunol. 2009;182:5914-8.

[260] Mselle TF, Howell AL, Ghosh M, Wira CR, Sentman CL. Human uterine natural killer cells but not blood natural killer cells inhibit human immunodeficiency virus type 1 infection by secretion of CXCL12. J Virol. 2009;83:11188-95.

[261] Boulet S, Sharafi S, Simic N, Bruneau J, Routy JP, Tsoukas CM, et al. Increased proportion of KIR3DS1 homozygotes in HIV-exposed uninfected individuals. AIDS. 2008;22:595-9.

[262] Alter G, Martin MP, Teigen N, Carr WH, Suscovich TJ, Schneidewind A, et al. Differential natural killer cell-mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes. J Exp Med. 2007;204:3027-36.

[263] Alter G, Rihn S, Walter K, Nolting A, Martin M, Rosenberg ES, et al. HLA class I subtype-dependent expansion of KIR3DS1+ and KIR3DL1+ NK cells during acute human immunodeficiency virus type 1 infection. J Virol. 2009;83:6798-805.

[264] Alter G, Rihn S, Streeck H, Teigen N, Piechocka-Trocha A, Moss K, et al. Ligand-independent exhaustion of killer immunoglobulin-like receptor-positive CD8+ T cells in human immunodeficiency virus type 1 infection. J Virol. 2008;82:9668-77.

[265] Long BR, Ndhlovu LC, Oksenberg JR, Lanier LL, Hecht FM, Nixon DF, et al. Conferral of enhanced natural killer cell function by KIR3DS1 in early human immunodeficiency virus type 1 infection. J Virol. 2008;82:4785-92.

[266] Bostik P, Kobkitjaroen J, Tang W, Villinger F, Pereira LE, Little DM, et al. Decreased NK cell frequency and function is associated with increased risk of KIR3DL

allele polymorphism in simian immunodeficiency virus-infected rhesus macaques with high viral loads. J Immunol. 2009;182:3638-49.

[267] O'Connell KA, Han Y, Williams TM, Siliciano RF, Blankson JN. Role of natural killer cells in a cohort of elite suppressors: low frequency of the protective KIR3DS1 allele and limited inhibition of human immunodeficiency virus type 1 replication in vitro. J Virol. 2009;83:5028-34.

[268] Thananchai H, Makadzange T, Maenaka K, Kuroki K, Peng Y, Conlon C, et al. Reciprocal recognition of an HLA-Cw4-restricted HIV-1 gp120 epitope by CD8+ T cells and NK cells. AIDS. 2009;23:189-93.

[269] Wong AH, Williams K, Reddy S, Wilson D, Giddy J, Alter G, et al. Alterations in Natural Killer Cell Receptor Profiles During HIV Type 1 Disease Progression Among Chronically Infected South African Adults. AIDS Res Hum Retrov. 2010.

[270] Schluns KS, Lefrancois L. Cytokine control of memory T-cell development and survival. Nat Rev Immunol. 2003;3:269-79.

[271] Hauser GJ, Bino T, Rosenberg H, Zakuth V, Geller E, Spirer Z. Interleukin-2 production and response to exogenous interleukin-2 in a patient with the acquired immune deficiency syndrome (AIDS). Clin Exp Immunol. 1984;56:14-7.

[272] Clerici M, Hakim F, Venzon D, Blatt S, Hendrix C, Wynn T, et al. Changes in interleukin-2 and interleukin-4 production in asymptomatic, human immunodeficiency virus-seropositive individuals. J Clin Invest. 1993;91:759-65.

[273] Clerici M, Shearer GM. A TH1-->TH2 switch is a critical step in the etiology of HIV infection. Immunol Today. 1993;14:107-11.

[274] David D, Bani L, Moreau JL, Treilhou MP, Nakarai T, Joussemet M, et al. Regulatory dysfunction of the interleukin-2 receptor during HIV infection and the impact of triple combination therapy. PNAS. 1998;95:11348-53.

[275] Kryworuchko M, Pasquier V, Keller H, David D, Goujard C, Gilquin J, et al. Defective interleukin-2-dependent STAT5 signalling in CD8 T lymphocytes from HIV-positive patients: restoration by antiretroviral therapy. AIDS. 2004;18:421-6.

[276] Clerici M, Shearer G. The Th1-Th2 hypothesis of HIV infection: new insights. Immunol Today. 1994;15:575-81.

[277] Lucey DR, Clerici M, Shearer GM. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. Clin Microbiol Rev. 1996;9:532-62.

[278] Fakoya A, Matear PM, Filley E, Rook GA, Stanford J, Gilson RJ, et al. HIV infection alters the production of both type 1 and 2 cytokines but does not induce a polarized type 1 or 2 state. AIDS. 1997;11:1445-52.

[279] Graziosi C, Pantaleo G, Gantt KR, Fortin JP, Demarest JF, Cohen OJ, et al. Lack of evidence for the dichotomy of TH1 and TH2 predominance in HIV-infected individuals. Science. 1994;265:248-52.

[280] Tanaka M, Hirabayashi Y, Gatanaga H, Aizawa S, Hachiya A, Takahashi Y, et al. Reduction in interleukin-2-producing cells but not Th1 to Th2 shift in moderate and advanced stages of human immunodeficiency virus type-1-infection: direct analysis of intracellular cytokine concentrations in CD4+ CD8- T cells. Scand J Immunol. 1999;50:550-4.

[281] Diaz-Mitoma F, Kumar A, Karimi S, Kryworuchko M, Daftarian MP, Creery WD, et al. Expression of IL-10, IL-4 and interferon-gamma in unstimulated and mitogen-stimulated peripheral blood lymphocytes from HIV-seropositive patients. Clin Exp Immunol. 1995;102:31-9.

[282] Levy JA. HIV pathogenesis and long-term survival. AIDS. 1993;7:1401-10.

[283] Vigano A, Principi N, Crupi L, Onorato J, Vincenzo ZG, Salvaggio A. Elevation of IgE in HIV-infected children and its correlation with the progression of disease. J Allergy Clin Immunol. 1995;95:627-32.

[284] Vigano A, Principi N, Villa ML, Riva C, Crupi L, Trabattoni D, et al. Immunologic characterization of children vertically infected with human immunodeficiency virus, with slow or rapid disease progression. J Pediatr. 1995;126:368-74.

[285] Israel-Biet D, Labrousse F, Tourani JM, Sors H, Andrieu JM, Even P. Elevation of IgE in HIV-infected subjects: a marker of poor prognosis. J Allergy Clin Immunol. 1992;89:68-75.

[286] Lucey DR, Zajac RA, Melcher GP, Butzin CA, Boswell RN. Serum IgE levels in 622 persons with human immunodeficiency virus infection: IgE elevation with marked depletion of CD4+ T-cells. AIDS Res Hum Retrov. 1990;6:427-9.

[287] Miguez-Burbano MJ, Shor-Posner G, Fletcher MA, Lu Y, Moreno JN, Carcamo C, et al. Immunoglobulin E levels in relationship to HIV-1 disease, route of infection, and vitamin E status. Allergy. 1995;50:157-61.

[288] Wright DN, Nelson RP, Ledford DK, Fernandez-Caldas E, Trudeau WL, Lockey RF. Serum IgE and human immunodeficiency virus (HIV) infection. J Allergy Clin Immunol. 1990;85:445-52.

[289] Becker Y. The changes in the T helper 1 (Th1) and T helper 2 (Th2) cytokine balance during HIV-1 infection are indicative of an allergic response to viral proteins that may be reversed by Th2 cytokine inhibitors and immune response modifiers--a review and hypothesis. Virus Genes. 2004;28:5-18.

[290] Mirza O, Henriksen A, Ipsen H, Larsen JN, Wissenbach M, Spangfort MD, et al. Dominant epitopes and allergic cross-reactivity: complex formation between a Fab fragment of a monoclonal murine IgG antibody and the major allergen from birch pollen Bet v 1. J Immunol. 2000;165:331-8.

[291] Horikoshi H, Kinomoto M, Kurosu T, Komoto S, Shiraga M, Otake T, et al. Resting CD4(+) T cells with CD38(+)CD62L(+) produce interleukin-4 which contributes to enhanced replication of T-tropic human immunodeficiency virus type 1. Virology. 2002;293:94-102.

[292] Do H, Vasilescu A, Diop G, Hirtzig T, Coulonges C, Labib T, et al. Associations of the IL2Ralpha, IL4Ralpha, IL10Ralpha, and IFN (gamma) R1 cytokine receptor genes with AIDS progression in a French AIDS cohort. Immunogenetics. 2006;58:89-98.

[293] Soriano A, Lozano F, Oliva H, Garcia F, Nomdedeu M, De Lazzari E, et al. Polymorphisms in the interleukin-4 receptor alpha chain gene influence susceptibility to HIV-1 infection and its progression to AIDS. Immunogenetics. 2005;57:644-54.

[294] Wang C, Song W, Lobashevsky E, Wilson CM, Douglas SD, Mytilineos J, et al. Cytokine and chemokine gene polymorphisms among ethnically diverse North Americans with HIV-1 infection. J Acquir Immune Defic Syndr. 2004;35:446-54.

[295] Benoit A, Abdkader K, Sirskyj D, Alhetheel A, Sant N, Diaz-Mitoma F, et al. Inverse association of repressor growth factor independent-1 with CD8 T cell interleukin (IL)-7 receptor [alpha] expression and limited signal transducers and activators of transcription signaling in response to IL-7 among [gamma]-chain cytokines in HIV patients. AIDS. 2009;23:1341-7.

[296] Napolitano LA, Grant RM, Deeks SG, Schmidt D, De Rosa SC, Herzenberg LA, et al. Increased production of IL-7 accompanies HIV-1-mediated T-cell depletion: implications for T-cell homeostasis. Nat Med. 2001;7:73-9.

[297] Rethi B, Fluur C, Atlas A, Krzyzowska M, Mowafi F, Grutzmeier S, et al. Loss of IL-7Ralpha is associated with CD4 T-cell depletion, high interleukin-7 levels and CD28 down-regulation in HIV infected patients. AIDS. 2005;19:2077-86.

[298] Boulassel MR, Young M, Routy JP, Sekaly RP, Tremblay C, Rouleau D. Circulating levels of IL-7 but not IL-15, IGF-1, and TGF-beta are elevated during primary HIV-1 infection. HIV Clin Trials. 2004;5:357-9.

[299] Sasson SC, Zaunders JJ, Zanetti G, King EM, Merlin KM, Smith DE, et al. Increased plasma interleukin-7 level correlates with decreased CD127 and Increased CD132 extracellular expression on T cell subsets in patients with HIV-1 infection. J Infect Dis. 2006;193:505-14.

[300] Albuquerque AS, Cortesao CS, Foxall RB, Soares RS, Victorino RM, Sousa AE. Rate of increase in circulating IL-7 and loss of IL-7Ralpha expression differ in HIV-1 and HIV-2 infections: two lymphopenic diseases with similar hyperimmune activation but distinct outcomes. J Immunol. 2007;178:3252-9.

[301] Fluur C, Rethi B, Thang PH, Vivar N, Mowafi F, Lopalco L, et al. Relationship between serum IL-7 concentrations and lymphopenia upon different levels of HIV immune control. AIDS. 2007;21:1048-50.

[302] Fluur C, De Milito A, Fry TJ, Vivar N, Eidsmo L, Atlas A, et al. Potential role for IL-7 in Fas-mediated T cell apoptosis during HIV infection. J Immunol. 2007;178:5340-50.

[303] Jaleco S, Swainson L, Dardalhon V, Burjanadze M, Kinet S, Taylor N. Homeostasis of naive and memory CD4+ T cells: IL-2 and IL-7 differentially regulate

the balance between proliferation and Fas-mediated apoptosis. J Immunol. 2003;171:61-8.

[304] Managlia EZ, Landay A, Al-Harthi L. Interleukin-7 signalling is sufficient to phenotypically and functionally prime human CD4 naive T cells. Immunology. 2005;114:322-35.

[305] Colle JH, Moreau JL, Fontanet A, Lambotte O, Joussemet M, Jacod S, et al. Regulatory dysfunction of the interleukin-7 receptor in CD4 and CD8 lymphocytes from HIV-infected patients--effects of antiretroviral therapy. J Acquir Immune Defic Syndr. 2006;42:277-85.

[306] MacPherson P, Fex C, Sanchez-Dardon J, Hawley-Foss N, Angel J. Interleukin-7 receptor expression on CD8(+) T cells is reduced in HIV infection and partially restored with effective antiretroviral therapy. J Acquir Immune Defic Syndr. 2001;28:454-7.

[307] Paiardini M, Cervasi B, Albrecht H, Muthukumar A, Dunham R, Gordon S, et al. Loss of CD127 expression defines an expansion of effector CD8+ T cells in HIV-infected individuals. J Immunol. 2005;174:2900-9.

[308] Boutboul F, Puthier D, Appay V, Pelle O, Ait-Mohand H, Combadiere B, et al. Modulation of interleukin-7 receptor expression characterizes differentiation of CD8 T cells specific for HIV, EBV and CMV. AIDS. 2005;19:1981-6.

[309] Koesters SA, Alimonti JB, Wachihi C, Matu L, Anzala O, Kimani J, et al. IL-7Ralpha expression on CD4+ T lymphocytes decreases with HIV disease progression and inversely correlates with immune activation. Eur J Immunol. 2006;36:336-44.

[310] Vingerhoets J, Bisalinkumi E, Penne G, Colebunders R, Bosmans E, Kestens L, et al. Altered receptor expression and decreased sensitivity of T-cells to the stimulatory cytokines IL-2, IL-7 and IL-12 in HIV infection. Immunol Lett. 1998;61:53-61.

[311] Faller E, Kakal J, Kumar R, MacPherson P. IL-7 and the HIV Tat protein act synergistically to down-regulate CD127 expression on CD8 T cells. Int Immunol. 2009;21:203-16.

[312] Faller E, McVey M, Kakal J, MacPherson P. Interleukin-7 receptor expression on CD8 T-cells is downregulated by the HIV Tat protein. J Acquir Immune Defic Syndr. 2007;43:257-69.

[313] Colle JH, Moreau JL, Fontanet A, Lambotte O, Delfraissy JF, Theze J. The correlation between levels of IL-7Ralpha expression and responsiveness to IL-7 is lost in CD4 lymphocytes from HIV-infected patients. AIDS. 2007;21:101-3.

[314] Vassena L, Proschan M, Fauci A, Lusso P. Interleukin 7 reduces the levels of spontaneous apoptosis in CD4+ and CD8+ T cells from HIV-1-infected individuals. PNAS. 2007;104:2355-60.

[315] Crawley AM, Faucher S, Angel JB. Soluble IL-7Ralpha (sCD127) inhibits IL-7 activity and is increased in HIV infection. J Immunol. 2010;184:4679-87.

[316] Pestka S, Krause CD, Sarkar D, Walter MR, Shi Y, Fisher PB. Interleukin-10 and related cytokines and receptors. Annu Rev Immunol. 2004;22:929-79.

[317] Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. J Immunol. 2008;180:5771-7.

[318] Mocellin S, Panelli MC, Wang E, Nagorsen D, Marincola FM. The dual role of IL-10. Trends Immunol. 2003;24:36-43.

[319] de Waal Malefyt R, Haanen J, Spits H, Roncarolo MG, te Velde A, Figdor C, et al. Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. J Exp Med. 1991;174:915-24.

[320] Liu Y, de Waal Malefyt R, Briere F, Parham C, Bridon JM, Banchereau J, et al. The EBV IL-10 homologue is a selective agonist with impaired binding to the IL-10 receptor. J Immunol. 1997;158:604-13.

[321] Kotenko SV, Saccani S, Izotova LS, Mirochnitchenko OV, Pestka S. Human cytomegalovirus harbors its own unique IL-10 homolog (cmvIL-10). PNAS. 2000;97:1695-700.

[322] Stylianou E, Aukrust P, Kvale D, Muller F, Froland SS. IL-10 in HIV infection: increasing serum IL-10 levels with disease progression--down-regulatory effect of potent anti-retroviral therapy. Clin Exp Immunol. 1999;116:115-20.

[323] Clerici M, Wynn TA, Berzofsky JA, Blatt SP, Hendrix CW, Sher A, et al. Role of interleukin-10 in T helper cell dysfunction in asymptomatic individuals infected with the human immunodeficiency virus. J Clin Invest. 1994;93:768-75.

[324] Brockman MA, Kwon DS, Tighe DP, Pavlik DF, Rosato PC, Sela J, et al. IL-10 is up-regulated in multiple cell types during viremic HIV infection and reversibly inhibits virus-specific T cells. Blood. 2009;114:346-56.

[325] Gee K, Angel JB, Mishra S, Blahoianu MA, Kumar A. IL-10 regulation by HIV-Tat in primary human monocytic cells: Involvement of calmodulin/calmodulin-dependent protein kinase-activated p38 MAPK and Sp-1 and CREB-1 transcription factors. J Immunol. 2007;178:798-807.

[326] Trinchieri G, Pflanz S, Kastelein RA. The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. Immunity. 2003;19:641-4.

[327] Rockstroh JK, Kreuzer KA, Sauerbruch T, Spengler U. Protein levels of interleukin-12 p70 holomer, its p40 chain and interferon-gamma during advancing HIV infection. J Infect. 1998;37:282-6.

[328] Denis M, Ghadirian E. Dysregulation of interleukin 8, interleukin 10, and interleukin 12 release by alveolar macrophages from HIV type 1-infected subjects. AIDS Res Hum Retrov. 1994;10:1619-27.

[329] Marshall JD, Chehimi J, Gri G, Kostman JR, Montaner LJ, Trinchieri G. The interleukin-12-mediated pathway of immune events is dysfunctional in human immunodeficiency virus-infected individuals. Blood. 1999;94:1003-11.

[330] Chambers KA, Parato KG, Angel JB. Active cellular infection of myeloid cells is required for HIV-1-mediated suppression of interleukin-12 p40 expression. Cell Immunol. 2002;215:120-32.

[331] Zou W, Lackner AA, Simon M, Durand-Gasselin I, Galanaud P, Desrosiers RC, et al. Early cytokine and chemokine gene expression in lymph nodes of macaques infected with simian immunodeficiency virus is predictive of disease outcome and vaccine efficacy. J Virol. 1997;71:1227-36.

[332] Ma W, Mishra S, Gajanayaka N, Angel JB, Kumar A. HIV-1 Nef inhibits lipopolysaccharide-induced IL-12p40 expression by inhibiting JNK-activated NFkappaB in human monocytic cells. J Biol Chem. 2009;284:7578-87.

[333] Ito M, Ishida T, He L, Tanabe F, Rongge Y, Miyakawa Y, et al. HIV type 1 Tat protein inhibits interleukin 12 production by human peripheral blood mononuclear cells. AIDS Res Hum Retrov. 1998;14:845-9.

[334] Mirani M, Elenkov I, Volpi S, Hiroi N, Chrousos GP, Kino T. HIV-1 protein Vpr suppresses IL-12 production from human monocytes by enhancing glucocorticoid action: potential implications of Vpr coactivator activity for the innate and cellular immunity deficits observed in HIV-1 infection. J Immunol. 2002;169:6361-8.

[335] Taoufik Y, Lantz O, Wallon C, Charles A, Dussaix E, Delfraissy JF. Human immunodeficiency virus gp120 inhibits interleukin-12 secretion by human monocytes: an indirect interleukin-10-mediated effect. Blood. 1997;89:2842-8.

[336] Kacani L, Stoiber H, Dierich MP. Role of IL-15 in HIV-1-associated hypergammaglobulinaemia. Clin Exp Immunol. 1997;108:14-8.

[337] Ahmad R, Sindhu STA, Toma E, Morisset R, Ahmad A. Studies on the production of IL-15 in HIV-infected/AIDS patients. J Clin Immunol. 2003;23:81-90.

[338] Ahmad A, Ahmad R, Toma E, Morisset R, Menezes J. Impaired induction of IL-15 in response to herpes simplex virus type 1 infection in peripheral blood mononuclear cells of HIV-infected patients. AIDS. 2000;14:744-6.

[339] Chehimi J, Marshall JD, Salvucci O, Frank I, Chehimi S, Kawecki S, et al. IL-15 enhances immune functions during HIV infection. J Immunol. 1997;158:5978-87.

[340] D'Ettorre G, Forcina G, Lichtner M, Mengoni F, D'Agostino C, Massetti A, et al. Interleukin-15 in HIV infection: immunological and virological interactions in antiretroviral-naive and -treated patients. AIDS. 2002;16:181-8.

[341] Appay V, Nixon DF, Donahoe SM, Gillespie GMA, Dong T, King A, et al. HIV-specific CD8+ T cells produce antiviral cytokines but are impaired in cytolytic function. J Exp Med. 2000;192:63-76.

[342] Champagne P, Ogg GS, King AS, Knabenhans C, Ellefsen K, Nobile M, et al. Skewed maturation of memory HIV-specific CD8 T lymphocytes. Nature. 2001;410:106-11.

[343] Brenchley JM, Karandikar NJ, Betts MR, Ambrozak DR, Hill BJ, Crotty LE, et al. Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. Blood. 2003;101:2711-20.

[344] Mueller YM, Bojczuk PM, Halstead ES, Kim AH, Witek J, Altman JD, et al. IL-15 enhances survival and function of HIV-specific CD8+ T cells. Blood. 2003;101:1024-9.

[345] Mueller YM, Makar V, Bojczuk PM, Witek J, Katsikis PD. IL-15 enhances the function and inhibits CD95/Fas-induced apoptosis of human CD4+ and CD8+ effector-memory T cells. Int Immunol. 2003;15:49-58.

[346] Agostini C, Trentin L, Sancetta R, Facco M, Tassinari C, Cerutti A, et al. Interleukin-15 triggers activation and growth of the CD8 T-cell pool in extravascular tissues of patients with acquired immunodeficiency syndrome. Blood. 1997;90:1115-23.

[347] Zaunders JJ, Moutouh-de Parseval L, Kitada S, Reed JC, Rought S, Genini D, et al. Polyclonal proliferation and apoptosis of CCR5+ T lymphocytes during primary human immunodeficiency virus type 1 infection: regulation by interleukin (IL)-2, IL-15, and Bcl-2. J Infect Dis. 2003;187:1735-47.

[348] Chae DW, Nosaka Y, Strom TB, Maslinski W. Distribution of IL-15 receptor alpha-chains on human peripheral blood mononuclear cells and effect of immunosuppressive drugs on receptor expression. J Immunol. 1996;157:2813-9.

[349] Pahwa R, McCloskey TW, Aroniadis OC, Strbo N, Krishnan S, Pahwa S. CD8+ T Cells in HIV disease exhibit cytokine receptor perturbation and poor T cell receptor activation but are responsive to gamma-chain cytokine-driven proliferation. J Infect Dis. 2006;193:879-87.

[350] Wiercinska-Drapalo A, Flisiak R, Jaroszewicz J, Prokopowicz D. Increased plasma transforming growth factor-b is associated with disease progression in HIV-1-infected patients. Viral Immunol. 2004;17:109-13.

[351] Brooks SP, Bernstein ZP, Schneider SL, Gollnick SO, Tomasi TB. Role of transforming growth factor-beta1 in the suppressed allostimulatory function of AIDS patients. AIDS. 1998;12:481-7.

[352] Grant RM, Hecht FM, Warmerdam M, Liu L, Liegler T, Petropoulos CJ, et al. Time trends in primary HIV-1 drug resistance among recently infected persons. JAMA. 2002;288:181-8.

[353] Parkin NT, Lie YS, Hellmann N, Markowitz M, Bonhoeffer S, Ho DD, et al. Phenotypic changes in drug susceptibility associated with failure of human immunodeficiency virus type 1 (HIV-1) triple combination therapy. J Infect Dis. 1999;180:865-70.

[354] Marchetti G, Franzetti F, Gori A. Partial immune reconstitution following highly active antiretroviral therapy: can adjuvant interleukin-2 fill the gap? J Antimicrob Chemother. 2005;55:401-9.

[355] Stacey AR, Norris PJ, Qin L, Haygreen EA, Taylor E, Heitman J, et al. Induction of a striking systemic cytokine cascade prior to peak viremia in acute human immunodeficiency virus type 1 infection, in contrast to more modest and delayed responses in acute hepatitis B and C virus infections. J Virol. 2009;83:3719-33.

[356] Lane HC, Siegel JP, Rook AH, Masur H, Gelmann EP, Quinnan GV, et al. Use of interleukin-2 in patients with acquired immunodeficiency syndrome. J Biol Response Mod. 1984;3:512-6.

[357] Pau AK, Tavel JA. Therapeutic use of interleukin-2 in HIV-infected patients. Curr Opin Pharmacol. 2002;2:433-9.

[358] Emery S, Capra WB, Cooper DA, Mitsuyasu RT, Kovacs JA, Vig P, et al. Pooled analysis of 3 randomized, controlled trials of interleukin-2 therapy in adult human immunodeficiency virus type 1 disease. J Infect Dis. 2000;182:428-34.

[359] David D, Keller H, Nait-Ighil L, Treilhou MP, Joussemet M, Dupont B, et al. Involvement of Bcl-2 and IL-2R in HIV-positive patients whose CD4 cell counts fail to increase rapidly with highly active antiretroviral therapy. AIDS. 2002;16:1093-101.

[360] David D, Nait-Ighil L, Dupont B, Maral J, Gachot B, Theze J. Rapid effect of interleukin-2 therapy in human immunodeficiency virus-infected patients whose CD4 cell counts increase only slightly in response to combined antiretroviral treatment. J Infect Dis. 2001;183:730-5.

[361] Dunn C, Chalupny NJ, Sutherland CL, Dosch S, Sivakumar PV, Johnson DC, et al. Human cytomegalovirus glycoprotein UL16 causes intracellular sequestration of NKG2D ligands, protecting against natural killer cell cytotoxicity. J Exp Med. 2003;197:1427-39.

[362] Mitsuyasu R, Gelman R, Cherng DW, Landay A, Fahey J, Reichman R, et al. The virologic, immunologic, and clinical effects of interleukin 2 with potent antiretroviral therapy in patients with moderately advanced human immunodeficiency virus infection: a randomized controlled clinical trial--AIDS Clinical Trials Group 328. Arch Intern Med. 2007;167:597-605.

[363] Youle M, Emery S, Fisher M, Nelson M, Fosdick L, Janossy G, et al. A randomised trial of subcutaneous intermittent interleukin-2 without antiretroviral therapy in HIV-infected patients: the UK-Vanguard Study. PLoS Clin Trials. 2006;1:e3.

[364] Lentsch AB, Miller FN, Edwards MJ. Mechanisms of leukocyte-mediated tissue injury induced by interleukin-2. Cancer Immunol Immunother. 1999;47:243-8.

[365] Locker GJ, Kapiotis S, Veitl M, Mader RM, Stoiser B, Kofler J, et al. Activation of endothelium by immunotherapy with interleukin-2 in patients with malignant disorders. Br J Haematol. 1999;105:912-9.

[366] Natarajan V, Lempicki RA, Sereti I, Badralmaa Y, Adelsberger JW, Metcalf JA, et al. Increased peripheral expansion of naive CD4+ T cells in vivo after IL-2 treatment of patients with HIV infection. PNAS. 2002;99:10712-7.

[367] Sereti I, Anthony KB, Martinez-Wilson H, Lempicki R, Adelsberger J, Metcalf JA, et al. IL-2-induced CD4+ T-cell expansion in HIV-infected patients is associated with long-term decreases in T-cell proliferation. Blood. 2004;104:775-80.

[368] Sereti I, Martinez-Wilson H, Metcalf JA, Baseler MW, Hallahan CW, Hahn B, et al. Long-term effects of intermittent interleukin 2 therapy in patients with HIV infection: characterization of a novel subset of CD4(+)/CD25(+) T cells. Blood. 2002;100:2159-67.

[369] Aandahl EM, Michaelsson J, Moretto WJ, Hecht FM, Nixon DF. Human CD4+ CD25+ regulatory T cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens. J Virol. 2004;78:2454-9.

[370] Kinter AL, Hennessey M, Bell A, Kern S, Lin Y, Daucher M, et al. CD25(+)CD4(+) regulatory T cells from the peripheral blood of asymptomatic HIV-infected individuals regulate CD4(+) and CD8(+) HIV-specific T cell immune responses in vitro and are associated with favorable clinical markers of disease status. J Exp Med. 2004;200:331-43.

[371] Weiss L, Donkova-Petrini V, Caccavelli L, Balbo M, Carbonneil C, Levy Y. Human immunodeficiency virus-driven expansion of CD4+CD25+ regulatory T cells, which suppress HIV-specific CD4 T-cell responses in HIV-infected patients. Blood. 2004;104:3249-56.

[372] Rosenberg SA, Sportes C, Ahmadzadeh M, Fry TJ, Ngo LT, Schwarz SL, et al. IL-7 administration to humans leads to expansion of CD8+ and CD4+ cells but a relative decrease of CD4+ T-regulatory cells. J Immunother. 2006;29:313-9.

[373] Levy Y. Enhanced T cell recovery in HIV-1-infected adults through IL-7 treatment. J Clin Invest. 2009;119:997-1007.

[374] Sportes C, Gress RE, Mackall CL. Perspective on potential clinical applications of recombinant human interleukin-7. Ann N Y Acad Sci. 2009;1182:28-38.

[375] Sportes C, Babb RR, Krumlauf MC, Hakim FT, Steinberg SM, Chow CK, et al. Phase I study of recombinant human interleukin-7 administration in subjects with refractory malignancy. Clin Cancer Res. 2010;16:727-35.

[376] Sportes C, Hakim FT, Memon SA, Zhang H, Chua KS, Brown MR, et al. Administration of rhIL-7 in humans increases in vivo TCR repertoire diversity by preferential expansion of naive T cell subsets. J Exp Med. 2008;205:1701-14.

[377] Sereti I, Dunham RM, Spritzler J, Aga E, Proschan MA, Medvik K, et al. IL-7 administration drives T cell cycle entry and expansion in HIV-1 infection. Blood. 2009;e-publised.

[378] Alpdogan O, van den Brink MR. IL-7 and IL-15: therapeutic cytokines for immunodeficiency. Trends Immunol. 2005;26:56-64.

[379] Munger W, DeJoy SQ, Jeyaseelan R, Sr., Torley LW, Grabstein KH, Eisenmann J, et al. Studies evaluating the antitumor activity and toxicity of interleukin-15, a new T cell growth factor: comparison with interleukin-2. Cell Immunol. 1995;165:289-93.

[380] Tsunobuchi H, Nishimura H, Goshima F, Daikoku T, Suzuki H, Nakashima I, et al. A protective role of interleukin-15 in a mouse model for systemic infection with herpes simplex virus. Virology. 2000;275:57-66.

[381] Ross ME, Caligiuri MA. Cytokine-induced apoptosis of human natural killer cells identifies a novel mechanism to regulate the innate immune response. Blood. 1997;89:910-8.

[382] Dao T, Ohashi K, Kayano T, Kurimoto M, Okamura H. Interferon-[gamma]-inducing factor, a novel cytokine, enhances Fas ligand-mediated cytotoxicity of murine T helper 1 cells. Cell Immunol. 1996;173:230-5.

[383] Hashimoto W, Osaki T, Okamura H, Robbins PD, Kurimoto M, Nagata S, et al. Differential antitumor effects of administration of recombinant IL-18 or recombinant IL-12 are mediated primarily by Fas-Fas ligand- and perforin-induced tumor apoptosis, respectively. J Immunol. 1999;163:583-9.

[384] Tsutsui H, Nakanishi K, Matsui K, Higashino K, Okamura H, Miyazawa Y, et al. IFN-gamma-inducing factor up-regulates Fas ligand-mediated cytotoxic activity of murine natural killer cell clones. J Immunol. 1996;157:3967-73.

[385] Tsutsui H, Matsui K, Kawada N, Hyodo Y, Hayashi N, Okamura H, et al. IL-18 accounts for both TNF-alpha- and Fas ligand-mediated hepatotoxic pathways in endotoxin-induced liver injury in mice. J Immunol. 1997;159:3961-7.

[386] Tsutsui H, Matsui K, Okamura H, Nakanishi K. Pathophysiological roles of interleukin-18 in inflammatory liver diseases. Immunol Rev. 2000;174:192-209.

[387] Hodge DL, Subleski JJ, Reynolds DA, Buschman MD, Schill WB, Burkett MW, et al. The proinflammatory cytokine interleukin-18 alters multiple signaling pathways to inhibit natural killer cell death. J Interf & Cytok Res. 2006;26:706-18.

[388] Mailliard RB, Alber SM, Shen H, Watkins SC, Kirkwood JM, Herberman RB, et al. IL-18-induced CD83+CCR7+ NK helper cells. J Exp Med. 2005;202:941-53.

[389] Ahmad R, Sindhu STA, Toma E, Morisset R, Ahmad A. Elevated Levels of Circulating Interleukin-18 in Human Immunodeficiency Virus-Infected Individuals: Role of Peripheral Blood Mononuclear Cells and Implications for AIDS Pathogenesis. J Virol. 2002;76:12448-56.

[390] Song W, Wilson CM, Allen S, Wang C, Li Y, Kaslow RA, et al. Interleukin 18 and human immunodeficiency virus type I infection in adolescents and adults. Clin Exp Immunol. 2006;144:117-24.

[391] Torre D, Pugliese A. Interleukin-18: a proinflammatory cytokine in HIV-1 infection. Curr HIV Res. 2006;4:423-30.

[392] Torre D, Speranza F, Martegani R, Pugliese A, Castelli F, Basilico C, et al. Circulating levels of IL-18 in adult and paediatric patients with HIV-1 infection. AIDS. 2000;14.

[393] Chen L, Jarujaron S, Wu X, Sun L, Zha W, Liang G, et al. HIV protease inhibitor lopinavir-induced TNF-alpha and IL-6 expression is coupled to the unfolded protein

response and ERK signaling pathways in macrophages. Biochem Pharmacol. 2009;78:70-7.

[394] Aquaro S, CaliÚ R, Balzarini J, Bellocchi MC, Garaci E, Perno CF. Macrophages and HIV infection: therapeutical approaches toward this strategic virus reservoir. Antiv Res. 2002;55:209-25.

[395] Sailer CA, Pott GB, Dinarello CA, Whinney SM, Forster JE, Larson-Duran JK, et al. Whole-blood interleukin-18 level during early HIV-1 infection is associated with reduced CXCR4 coreceptor expression and interferon-[gamma] levels. J Infect Dis. 2007;195:734-8.

[396] Hurgin V, Novick D, Rubinstein M. The promoter of IL-18 binding protein: activation by an IFN-gamma -induced complex of IFN regulatory factor 1 and CCAAT/enhancer binding protein beta. PNAS. 2002;99:16957-62.

[397] Koirala J, Adamski A, Koch L, Stueber D, El-Azizi M, Khardori NM, et al. Interferon-gamma receptors in HIV-1 infection. AIDS Res and Hum Retrov. 2008;0:1-6.

[398] Iannello A, Debbeche O, Samarani S, Ahmad A. Antiviral NK cell responses in HIV infection: II. viral strategies for evasion and lessons for immunotherapy and vaccination. J Leukoc Biol. 2008;84:27-49.

[399] Iannello A, Tremblay C, Routy J, Boulassel M, Toma E, Ahmad A. Decreased levels of circulating IL-21 in HIV-infected AIDS patients: Correlation with CD4+ T cell counts. Viral Immunol. 2008;21:385-8.

[400] Allen J, Wong H, Guyre P, Simon G, Wahl S. Association of circulating receptor Fc gamma RIII-positive monocytes in AIDS patients with elevated levels of transforming growth factor-beta. J Clin Invest. 1991;87:1773-9.

[401] Lotz M, Seth P. TGF beta and HIV infection. Ann N Y Acad Sci. 1993;685:501-11.

[402] Parato K, Kumar A, Badley A, Sanchez-Dardon J, Chambers K, Young C, et al. Normalization of natural killer cell function and phenotype with effective anti-HIV therapy and the role of IL-10. AIDS. 2002;16:1251-6.

[403] Torre D. Is interleukin-18 a proviral or antiviral cytokine in HIV-1 infection? J Infect Dis. 2007;196:803-4.

[404] Klein S, Klebba C, Kauschat D, Pape M, Ozmen L, Hoelzer D, et al. Interleukin-18 stimulates HIV-1 replication in a T-cell line. Eur Cytok Netw. 2000;11:47-52.

[405] Pugliese A, Gennero L, Vidotto V, Speranza F, Tambini R, Torre D. Interleukin-18 enhances HIV-1 production in a human chronically-infected T cell line (H9-V). Cell Biochem and Funct. 2002;20:333-7.

[406] Choi HJ, Dinarello CA, Shapiro L. Interleukin-18 inhibits human immunodeficiency virus type 1 production in peripheral blood mononuclear cells. J Infect Dis. 2001;184:560-8.

[407] Stylianou E, Bjerkeli V, Yndestad A, Heggelund L, Waehre T, Damas JK, et al. Raised serum levels of interleukin-18 is associated with disease progression and may contribute to virological treatment failure in HIV-1-infected patients. Clin Exp Immunol. 2003;132:462-6.

[408] Wiercinska-Drapalo A, Jaroszewicz J, Flisiak R, Prokopowicz D. Plasma interleukin-18 is associated with viral load and disease progression in HIV-1-infected patients. Microb and Infect. 2004;6:1273-7.

[409] Iannello A, Samarani S, Debbeche O, Boulassel M, Tremblay C, Toma E, et al. Potential role of IL-18 in the immunopathogenesis of AIDS, HIV-associated lipodystrophy and related clinical conditions. Curr HIV Res. 2010;8:147-64.

[410] Wood IS, Wang B, Jenkins JR, Trayhurn P. The pro-inflammatory cytokine IL-18 is expressed in human adipose tissue and strongly upregulated by TNF[alpha] in human adipocytes. Biochem and Biophys Res Commun. 2005;337:422-9.

[411] Lindegaard B, Hansen A, Gerstoft J, Pedersen B. High plasma level of interleukin-18 in HIV-infected subjects with lipodystrophy. J Acquir Immune Defic Syndr. 2004;36:588-93.

[412] Lindegaard B, Hansen A, Pilegaard H, Keller P, Gerstoft J, Pedersen B. Adipose tissue expression of IL-18 and HIV-associated lipodystrophy. AIDS. 2004;18:1956-8.

[413] Villarroya F, Domingo P, Giralt M. Lipodystrophy in HIV 1-infected patients: lessons for obesity research. Int J Obes. 2007;31:1763-9.

[414] Aso Y, Okumura K-i, Takebayashi K, Wakabayashi S, Inukai T. Relationships of plasma interleukin-18 concentrations to hyperhomocysteinemia and carotid intimal-media wall thickness in patients with type 2 diabetes. Diabetes Care. 2003;26:2622-7.

[415] Bruun JM, Stallknecht B, Helge JW, Richelsen B. Interleukin-18 in plasma and adipose tissue: effects of obesity, insulin resistance, and weight loss. Eur J Endocrinol. 2007;157:465-71.

[416] Gerdes N, Sukhova GK, Libby P, Reynolds RS, Young JL, Schonbeck U. Expression of interleukin (IL)-18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells, and macrophages: Implications for artherogenesis. J Exp Med. 2002;195:245-57.

[417] Mallat Z, Corbaz A, Scoazec A, Besnard S, Leseche G, Chvatchko Y, et al. Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. Circulation. 2001;104:1598-603.

[418] Mallat Z, Corbaz A, Scoazec A, Graber P, Alouani S, Esposito B, et al. Interleukin-18/Interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability. Circ Res. 2001;89:e41-5.

[419] Nakamura A, Shikata K, Hiramatsu M, Nakatou T, Kitamura T, Wada J, et al. Serum interleukin-18 levels are associated with nephropathy and atherosclerosis in japanese patients with type 2 diabetes. Diabetes Care. 2005;28:2890-5.

[420] Netea MG, Joosten LAB, Lewis E, Jensen DR, Voshol PJ, Kullberg BJ, et al. Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. Nat Med. 2006;12:650-6.

[421] Ances BM, Ellis RJ. Dementia and neurocognitive disorders due to HIV-1 infection. Semin Neurol. 2007;27:086-92.

[422] Aquaro S, Svicher V, Ronga L, Perno C, Pollicita M. HIV-1-associated dementia during HAART therapy. Recent Pat CNS Drug Discov. 2008;3:23-33.

[423] Brabers N, Nottet H. Role of the pro-inflammatory cytokines TNF-alpha; and IL-1beta; in HIV-associated dementia. Eur J of Clin Invest. 2006;36:447-58.

[424] Brazille P, Dereuddre-Bosquet N, Leport C, Clayette P, Boyer O, Dormont D, et al. Decreases in plasma TNF-alpha level and IFN-gamma mRNA level in peripheral blood mononuclear cells (PBMC) and an increase in IL-2 mRNA level in PBMC are associated with effective highly active antiretroviral therapy in HIV-infected patients. Clin & Exp Immunol. 2003;131:304-11.

[425] Clerici M, Balotta C, Meroni L, Ferrario E, Riva C, Trabattoni D, et al. Type 1 cytokine production and low prevalence of viral isolation correlate with long-term nonprogression in HIV infection. AIDS Res Hum Retrov. 1996;12:1053-61.

[426] D'Ettorre G, Forcina G, Andreotti M, Sarmati L, Palmisano L, Andreoni M, et al. Interleukin-15 production by monocyte-derived dendritic cells and T cell proliferation in HIV-infected patients with discordant response to highly active antiretroviral therapy. Clin & Exp Immunol. 2004;135:280-5.

[427] Isgrò A, Aiuti A, Mezzaroma I, Addesso M, Riva E, Giovannetti A, et al. Improvement of interleukin 2 production, clonogenic capability and restoration of stromal cell function in human immunodeficiency virus-type-1 patients after highly active antiretroviral therapy. Br J Haematol. 2002;118:864-74.

[428] Moanna A, Dunham R, Paiardini M, Silvestri G. CD4+ T-cell depletion in HIV infection: killed by friendly fire? Curr HIV/AIDS Rep. 2005;2:16-23.

[429] Ho IC, Lo D, Glimcher LH. c-maf promotes T helper cell type 2 (Th2) and attenuates Th1 differentiation by both interleukin 4-dependent and -independent mechanisms. J Exp Med. 1998;188:1859-66.

[430] Estaquier J, Idziorek T, de Bels F, Barré-Sinoussi F, Hurtrel B, Aubertin A, et al. Programmed cell death and AIDS: significance of T-cell apoptosis in pathogenic and nonpathogenic primate lentiviral infections. PNAS. 1994;91:9431-5.

[431] Naora H, Gougeon M. Enhanced survival and potent expansion of the natural killer cell population of HIV-infected individuals by exogenous interleukin-15. Immunol Lett. 1999;68:359-67.

[432] Pantaleo G, Graziosi C, Demarest J, Butini L, Montroni M, Fox C, et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature. 1993;362:355-8.

[433] Aiuti F, Mezzaroma I. Failure to reconstitute CD4+ T-cells despite suppression of HIV replication under HAART. AIDS Rev. 2006;8:88-97.

[434] Imami N, Hardy G, Pires A, Burton C, Pido-Lopez J, Mela C, et al. Immune reconstitution in HIV-1-infected patients. Curr Opin Investig Drugs. 2002;3:1138-45.

[435] Pahwa S. Role of common gamma chain utilizing cytokines for immune reconstitution in HIV infection. Immunol Res. 2007;38:373-86.

[436] Ostiguy V, Allard E-L, Marquis M, Leignadier J, Labrecque N. IL-21 promotes T lymphocyte survival by activating the phosphatidylinositol-3 kinase signaling cascade. J Leukoc Biol. 2007;82:645-56.

[437] Wilk E, Kalippke K, Buyny S, Schmidt R, Jacobs R. New aspects of NK cell subset identification and inference of NK cells' regulatory capacity by assessing functional and genomic profiles. Immunobiology. 2008;213:271-83.

[438] Iannello A, Ahmad A. Role of antibody-dependent cell-mediated cytotoxicity in the efficacy of therapeutic anti-cancer monoclonal antibodies. Cancer Metastasis Rev. 2005;24:487-99.

[439] Bolesta E, Kowalczyk A, Wierzbicki A, Eppolito C, Kaneko Y, Takiguchi M, et al. Increased level and longevity of protective immune responses induced by DNA vaccine expressing the HIV-1 Env glycoprotein when combined with IL-21 and IL-15 gene delivery. J Immunol. 2006;177:177-91.

[440] Gowda A, Roda J, Hussain S-RA, Ramanunni A, Joshi T, Schmidt S, et al. IL-21 mediates apoptosis through up-regulation of the BH3 family member BIM and enhances both direct and antibody-dependent cellular cytotoxicity in primary chronic lymphocytic leukemia cells in vitro. Blood. 2008;111:4723-30.

[441] Roda J, Joshi T, Butchar J, McAlees J, Lehman A, Tridandapani S, et al. The activation of natural killer cell effector functions by cetuximab-coated, epidermal growth factor receptor positive tumor cells is enhanced by cytokines. Clin Cancer Res. 2007;13:6419-28.

[442] Roda JM, Parihar R, Lehman A, Mani A, Tridandapani S, Carson WE, III. Interleukin-21 enhances NK cell activation in response to antibody-coated targets. J Immunol. 2006;177:120-9.

[443] Strbo N, de Armas L, Liu H, Kolber M, Lichtenheld M, Pahwa S. IL-21 augments natural killer effector functions in chronically HIV-infected individuals. AIDS. 2008;22:1551-60.

[444] van Leeuwen EM, Gamadia LE, Baars PA, Remmerswaal EB, ten Berge IJ, van Lier RA. Proliferation requirements of cytomegalovirus-specific, effector-type human CD8+ T cells. J Immunol. 2002;169:5838-43.

[445] Ahmad A, Ahmad R, Iannello A, Toma E, Morisset R, Sindhu S. IL-15 and HIV infection: lessons for immunotherapy and vaccination. Curr HIV Res. 2005;3:261-70.

[446] Bayard-McNeeley M, Doo H, He S, Hafner A, Johnson WD, Jr., Ho JL. Differential effects of interleukin-12, interleukin-15, and interleukin- 2 on human immunodeficiency virus type 1 replication in vitro. Clin Diagn Lab Immunol. 1996;3:547-53.

[447] Patki A, Quiñones-Mateu M, Dorazio D, Yen-Lieberman B, Boom W, Thomas E, et al. Activation of antigen-induced lymphocyte proliferation by interleukin-15 without the mitogenic effect of interleukin-2 that may induce human immunodeficiency virus-1 expression. J Clin Invest. 1996;98:616-21.

[448] Smithgall MD, Wong JG, Critchett KE, Haffar OK. IL-7 up-regulates HIV-1 replication in naturally infected peripheral blood mononuclear cells. J Immunol. 1996;156:2324-30.

[449] Fogli M, Mavilio D, Brunetta E, Varchetta S, Ata K, Roby G, et al. Lysis of endogenously infected CD4+ T cell blasts by rIL-2 activated autologous natural killer cells from HIV-infected viremic individuals. PLoS Pathog. 2008;4:e1000101.

[450] Barber DF, Faure M, Long EO. LFA-1 contributes an early signal for NK cell cytotoxicity. J Immunol. 2004;173:3653-9.

[451] Liu D, Bryceson YT, Meckel T, Vasiliver-Shamis G, Dustin ML, Long EO. Integrin-dependent organization and bidirectional vesicular traffic at cytotoxic immune synapses. Immunity. 2009;31:99-109.

[452] Perez OD, Mitchell D, Jager GC, Nolan GP. LFA-1 signaling through p44/42 is coupled to perforin degranulation in CD56+CD8+ natural killer cells. Blood. 2004;104:1083-93.

[453] Hioe CE, Chien PC, Jr., Lu C, Springer TA, Wang X-H, Bandres J, et al. LFA-1 expression on target cells promotes human immunodeficiency virus type 1 infection and transmission. J Virol. 2001;75:1077-82.

[454] Rodrigues L, Nandakumar S, Bonorino C, Rouse BT, Kumaraguru U. IL-21 and IL-15 cytokine DNA augments HSV specific effector and memory CD8+ T cell response. Mol Immunol. 2009;46:1494-504.

[455] Cui FD, Asada H, Jin ML, Kishida T, Shin-Ya M, Nakaya T, et al. Cytokine genetic adjuvant facilitates prophylactic intravascular DNA vaccine against acute and latent herpes simplex virus infection in mice. Gene Ther. 2005;12:160-8.

[456] van Leeuwen EM, van Buul JD, Remmerswaal EB, Hordijk PL, ten Berge IJ, van Lier RA. Functional re-expression of CCR7 on CMV-specific CD8+ T cells upon antigenic stimulation. Int Immunol. 2005;17:713-9.

[457] Hashmi MH, Van Veldhuizen PJ. Interleukin-21: updated review of Phase I and II clinical trials in metastatic renal cell carcinoma, metastatic melanoma and relapsed/refractory indolent non-Hodgkin's lymphoma. Expert Opin Biol Ther. 2010;10:807-17.

[458] Rasmussen MA, Colding-Jorgensen M, Hansen LT, Bro R. Multivariate evaluation of pharmacological responses in early clinical trials - a study of rIL-21 in the treatment of patients with metastatic melanoma. Br J Clin Pharmacol. 2010;69:379-90.

[459] Skak K, Kragh M, Hausman D, Smyth MJ, Sivakumar PV. Interleukin 21: combination strategies for cancer therapy. Nat Rev Drug Discov. 2008;7:231-40.

## 17- Articles non inclus dans la thèse de doctorat

Lors de cette thèse de doctorat, nous avons eu l'occasion de publier plusieurs autres articles de revues ainsi que des articles présentant des résultats originaux qui ne sont pas inclus dans cette thèse. Tous ces articles ont été soumis à un processus de révision par les pairs avant leur publication.

**Liste des articles** :

1. **Alexandre Iannello**, Olfa Debbeche, Raoudha El Arabi, Suzanne Samarani, David Hamel, Flore Rozenberg, Nikolaus Heveker, and Ali Ahmad. The HSV-1-induced FasL expression in human monocytic cells and its implications for cell death, viral replication and immune evasion. 2010. ***Viral Immunology***. (***Sous presse***)

2. **Alexandre Iannello,** Suzanne Samarani, Ali Ahmad. Comment on "HIV-specific IL-21 producing $CD4^+$ T cells are induced in acute and chronic progressive HIV infection and are associated with relative viral control". 2010. ***Journal of Immunology***. (***Sous presse***)

3. **Alexandre Iannello**, Volkan Seyrantepe, Feng Liang, Evgeny Kanshin, Preethi Jayanth, Myron R. Szewczuk, Suzanne Samarani, Ali Ahmad, and Alexey V. Pshezhetsky. Regulation of phagocytosis in macrophages by the NEU1 sialidase. 2010. ***Journal of Biological Chemistry*** **285(1): 206-15.** ***(Co-premier auteur)***

4. **Alexandre Iannello**, Olfa Debbeche, Suzanne Samarani, Sébastien Sabbagh, Michel Duval and Ali Ahmad. Pivotal role for NK cells in Future anti-tumoral immunotherapies ? 2006. ***Médecine Sciences*** **23(5): 502-508**.

5. **Alexandre Iannello**, Olfa Debbeche, Elodie Martin, Lynda Atallah, Suzanne Samarani and Ali Ahmad. Viral Strategies for Evading Anti-viral Cellular Immune Response of the Host. 2006. ***Journal of Leukocyte Biology*** **79(1): 16-35**.

6. Rasheed Ahmad, **Alexandre Iannello**, Suzanne Samarani, Richard Morisset, Emil Toma, Marianna Grosly and Ali Ahmad. Contribution of platelet activation to plasma IL-18 concentrations in HIV-infected AIDS patients. 2006. ***AIDS*** **20(14): 1907-9**.

7. **Alexandre Iannello** and Ali Ahmad. Role of antibody-dependent cell-mediated cytotoxicity in the efficacy of therapeutic anti-cancer monoclonal antibodies. 2005. ***Cancer and Metastasis Reviews*** **24(4): 483-495**.

8. Ali Ahmad, Rasheed Ahmad, **Alexandre Iannello**, Emil Toma, Richard Morisset, Sardar T.A.K. Sindhu. IL-15 and HIV infection: Lessons for immunotherapy and vaccination. 2005. ***Current HIV Research*** **3: 261-70**.