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Mini Review

The Effect of Interleukin-16 and its Precursor on T Lymphocyte Activation and Growth

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Interleukin-16 (IL-16) was the first described T lymphocyte chemoattractant. It has since been shown that IL-16 also functions as a primer of T cell proliferation, a modulator of inflammatory and immune responses, a stimulus of B cell differentiation and an inhibitor of Human immunodeficiency virus (HIV) replication. Its precursor, Prointerleukin-16 (pro-IL-16), is expressed in both the nucleus and cytoplasm of T cells. Cytoplasmic pro-IL-16 serves as the precursor for mature IL-16 while nuclear pro-IL-16 is associated with G0/G1 cell cycle arrest. Herein, we review the ability of IL-16 to act as both primer and modulator of T lymphocyte growth. The impact of IL-16 on T cell apoptosis is also discussed. Finally, we describe the role of pro-IL-16 as a T lymphocyte cell cycle growth suppressor.

Keywords: Interleukin-16; T lymphocyte growth; Precursor; Prointerleukin-16

INTRODUCTION

Interleukin-16 (IL-16) was initially described in 1982 as the first T cell chemoattractant (Center and Cruikshank, 1982; Cruikshank and Center, 1982). Designated lymphocyte chemoattractant factor (LCF), prior to its re-naming as IL-16 in 1994, the protein was identified in the supernatants of mitogen- or antigen-stimulated human peripheral blood mononuclear cells (PBMC). As such, it gained acceptance as a pro-inflammatory cytokine. Early beliefs that IL-16 is a pro-inflammatory cytokine were further supported by elucidation of its ability to induce IL-2Rα (CD25) expression (Cruikshank *et al.*, 1987) and prime T cell proliferation (Parada *et al.*, 1998). More recently, it has been recognized that IL-16 also functions as an immunomodulator, impairing antigen-induced T lymphocyte activation and proliferation (DeBie *et al.*, 2002; Little *et al.*, 2003; Lynch *et al.*, 2003a).

Pro-IL-16 protein exists as a 636 amino acid precursor which is detected in both the cytoplasm and nucleus. A larger, 1244 amino acid family member has also been identified exclusively in neuronal cells (Kurschner and Yuzaki, 1999). The two proteins are identical in their C-terminal 636 amino acids and appear to be products of alternative splicing.

In T lymphocytes, pro-IL-16 is constitutively expressed. In CD8+ T cells, caspase 3 constitutively cleaves pro-IL-16, releasing a 121 amino acid C-terminal fragment. Activation is required for caspase-3 cleavage of pro-IL-16 in CD4+ T cells. The C-terminal fragment

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autoaggregates and then is secreted as mature, bioactive IL-16 (Zhang *et al.*, 1998). Nuclear pro-IL-16 is associated with stabilization of p27kip protein and maintenance of T cells in G0/G1 cell cycle arrest (Zhang *et al.*, 2001; Wilson *et al.*, 2002).

Secreted IL-16 is a ligand for CD4. It has been shown to affect T cell activation and proliferation. IL-16 is the second cytokine whose secreted and precursor forms of protein have been shown to regulate cell activation and cell growth.

Herein, we review the impact of both IL-16 and pro-IL-16 on T lymphocyte activation and growth. We begin by discussing the direct growth enhancing effects of IL-16, its role in regulating T cell activation and apoptosis mediated by the T cell receptor (TcR) and its effect on Human immunodeficiency virus (HIV) replication. Finally, we discuss the recently described ability of nuclear pro-IL-16 to induce T cell cycle arrest.

While this chapter will focus on the ability of IL-16 and pro-IL-16 to alter T cell growth and activation, it must be noted that IL-16 protein has been detected in a number of other cell types. Other cells capable of generating IL-16 include, but are not limited to, eosinophils (Lim *et al.*, 1996), dendritic cells (Kaser *et al.*, 1999), B lymphocytes (Sharma *et al.*, 2000), airway epithelium (Little *et al.*, 2001), mast cells (Rumsaeng *et al.*, 1997), fibroblasts (Sciaky *et al.*, 2001) and neronal cells (Kurschner and Yuzaki, 1999). Though the effects of IL-16 and pro-IL-16 on cell activation and cell cycle have been best described in T lymphocytes, the potential exists that a similar effect may also occur in these other cell types.

IL-16 IS A PRIMER OF T CELL PROLIFERATION

The observation that IL-16 selectively induces chemotaxis of CD4+ T lymphocytes by direct interaction with CD4 and initiation of CD4-specific signaling led investigators to seek a potential role for IL-16 in the activation of CD4+ T lymphocytes. They found that IL-16 treatment of human peripheral CD4+ T lymphocytes induces expression of IL-2Rα (CD25) and IL-2Rβ, but not IL-2Rγ, in 24 h, consistent with cell activation (Cruikshank *et al.*, 1987). IL-16-induced expression of IL-2Rα (CD25) is detected in only 20–25% of CD4+ T cells; therefore, it may occur in a subset of CD4+, IL-16-responsive cells. The functional significance of this observation was confirmed by co-culture of resting human PBMCs with IL-16 and either IL-2 or IL-15. While IL-16 alone does not elicit an increase in thymidine uptake, there is marked enhancement of IL-2 or IL-15-induced thymidine uptake and expansion of CD4+ T cell numbers when IL-16 is present, compared to either IL-2 or IL-15 alone (Parada *et al.*, 1998).

IL-16's function as a primer of IL-2-dependent T lymphocyte proliferation has important clinical implications. Microarray experiments using lichen sclerosus atrophicus (LSA) tumor cells have demonstrated increased expression of both IL-2 and IL-16, suggesting that co-expression may be associated with uncontrolled cell growth in some cell types (Lombard *et al.*, 2003). Another example of the potential growth effect of IL-16 is found in Mycosis Fungoides, a cutaneous T cell lymphoma. Blaschke and coworkers have shown that IL-16 can be detected in the skin of healthy volunteers, in the uninvolved skin of Mycosis Fungoides patients, and in the lesions of Mycosis Fungoides patients (Blaschke *et al.*, 1999; Asadullah *et al.*, 2000). In contrast, IL-2 expression is rare in the skin of healthy volunteers and in the uninvolved skin of Mycosis Fungoides patients. However, IL-2 is readily detected in the lesions of Mycosis Fungoides patients. Since co-expression of IL-16 and IL-2 is present primarily in lesions of Mycosis Fungoides, the investigators analyzed whether a correlation existed between IL-16 and severity of the lesion. Their studies indicate a direct relationship between the amount of IL-16 expression and the severity of the lesion. Immunohistochemistry and *in situ* hybridization confirmed that infiltrating CD4+ T lymphocytes are the primary source of IL-16 (Blaschke *et al.*, 1999; Asadullah *et al.*, 2000).

Human T cell leukemia virus type 1 (HTLV-1)-associated myopathy / tropical spastic paraparesis (HAM/TSP) is a chronic, progressive neurologic disease characterized by degeneration of the spinal cord and the presence of infiltrating CD8+ T lymphocytes and macrophages. Biddison *et al.* have shown that HTLV-1 positive CD8+ T lymphocyte clones from the peripheral blood of HAM/TSP patients overexpress IL-16 (Biddison *et al.*, 1997). However, the cells do not secrete IL-2 and cannot expand in the absence of IL-2 as they rely on IL-2 secreted from local CD4+ T lymphocytes. While the role of IL-16 in this disease has not been determined, Biddison's study suggests that IL-16 may be involved in the pathogenesis on two separate levels: (a) chemoattraction of IL-2 secreting CD4+ T cells and (b) priming the CD4+ cells for IL-2-dependent cell expansion. This would result in IL-2-induced clonal expansion of HTLV-1 positive CD8+ T lymphocytes and non-clonal expansion of IL-2 producing CD4+ T lymphocytes.

IL-16 MODULATES ANTIGEN-INDUCED T LYMPHOCYTE ACTIVATION AND GROWTH

The initial bioactivities ascribed to IL-16 were chemoattraction of CD4+ T lymphocytes and upregulation of the IL-2 receptor, thus priming responding cells for IL-2 or IL-15 dependent proliferation. Based on these *in vitro* findings, IL-16 was initially classified as a pro-inflammatory cytokine. Since allergic diseases are examples of CD4+ T cell mediated inflammatory conditions, early studies sought to identify a relationship between IL-16 and atopy. In 1995, Cruikshank *et al.* detected an increased quantity of IL-16 in the bronchoalveolar lavage fluid (BALF) of antigen-challenged asthmatic subjects (Cruikshank *et al.*, 1995). Laberge and coworkers then used *in situ* hybridization and

immunohistochemistry to detect increased IL-16 mRNA and protein in bronchial biopsies and nasal biopsies from asthmatic and rhinitic subjects, respectively (Laberge *et al.*, 1997a,b). The primary cell of origin in both cases was the epithelial cell although IL-16 positive mononuclear cells were also present. These observations in humans, in combination with the *in vitro* pro-inflammatory bioactivities, led to animal studies designed to determine if IL-16 plays a pathogenic role in allergic airway inflammation.

As in humans, elevated levels of IL-16 were detected in the BALF of mice sensitized and aerosol-challenged with ovalbumin (Hessel *et al.*, 1998). The lung tissue was also noted to express high levels of IL-16 compared to unsensitized or sensitized, saline-challenged controls (Hessel *et al.*, 1998). Following intraperitoneal (i.p.) or intratracheal (i.t.) administration, the levels of IL-16 were further increased.

With these studies as background, De Bie and coworkers administered IL-16 to mice by i.p. injection and then characterized the antigen-induced inflammatory response. Surprisingly, they found that IL-16 completely inhibited antigen-induced airway hyperresponsiveness (AHR) and markedly decreased the number of eosinophils in the BALF and airway tissue. Furthermore, upon antigenic restimulation *in vitro*, there was reduced T lymphocyte proliferation and Th2-type cytokine production (DeBie *et al.*, 2002). When IL-16 was administered i.t. prior to methacholine challenge, ovalbumin induced airway hyperreactivity was markedly reduced (Little *et al.*, 2003). This suggests that the purpose of the elevated IL-16 protein in the airways is to downregulate antigen-driven Th2 cell-mediated inflammation and not a cause of inflammation as initially suspected.

Re-examination of *in vitro* bioactivities indicated that IL-16 pre-stimulation renders T cells unresponsive to stimulation by antigen or anti-CD3 antibody and markedly inhibits TcR dependent IL-2Rα expression (Cruikshank *et al.*, 1996; Theodore *et al.*, 1996). Additionally, it has been determined that IL-16 has differential effects on T cell subsets. Pinsonneault *et al.* found that secretion of Th2 cytokines, but not Th1 cytokines, following antigenic stimulation of T cells from atopic individuals was blocked by IL-16 (Pinsonneault *et al.*, 2001). Lynch *et al.* used a murine model to demonstrate that IL-16 is a more effective chemoattractant for Th1 than Th2 cells (Lynch *et al.*, 2003b). The finding that IL-16 affects Th1 and Th2 cells differently suggests that with preferential attraction of Th1 cells combined with selective inhibition of Th2 cytokine production, the responding T cell population will be significantly skewed towards a Th1 response. This concept is consistent with IL-16's described effect on asthmatic inflammation. In addition, several studies identifying that treatment with anti-IL-16 antibody attenuates disease parameters in Th1 diseases such as Crohn's disease (Keates *et al.*, 2000), delayed type hypersensitivity (Yoshimoto *et al.*, 2000) and Type I diabetes (Mi *et al.*, 2003), suggesting a pro-inflammatory effect in these diseases.

The receptor for IL-16, CD4, functions as a co-receptor for the TcR. Dependence on the presence and function of CD4 for maximal TcR signaling and cell activation has been well described. Ligation of CD4 by either antibody, HIV-1 gp120 (Chirmule *et al.*, 1990; Cefai *et al.*, 1990; Cefai *et al.*, 1992), or IL-16 (Cruikshank *et al.*, 1996; Theodore *et al.*, 1996) results in disruption of normal CD4 function and reduced T cell activation. The mechanism by which IL-16 disrupts CD4's contribution to TcR signaling is under investigation. These studies should contribute to our understanding of the differential effect of IL-16 on Th1 versus Th2 cells.

To confirm the immunomodulatory effect of IL-16 in allergic diseases, an IL-16 knock-out mouse has been developed, which has no overt, unstressed, phenotype. However, preliminary experiments demonstrate that ovalbumin sensitized IL-16 knock out mice demonstrate an increase in airway inflammation and hyperreactivity when exposed to aerosolized ovalbumin (Cruikshank, unpublished observations). The augmented inflammation observed in the absence of IL-16 indicates that endogenous IL-16, generated in association with allergenic stimulation, functions to regulate the inflammatory response.

In addition to affecting TcR-mediated T cell activation, IL-16 regulates T cell migration induced by some chemokines. IL-16 is a direct CD4+ T lymphocyte chemoattractant; however, it also has indirect inhibitory effects on chemoattraction induced by ligands of CCR5 (Mashikian *et al.*, 1999) and CXCR4 (MIP-1β and SDF-1α, respectively) (Van Drenth *et al.*, 2000). CCR5 is constitutively associated with CD4. CXCR4 can be induced to associate with CD4 following IL-16 stimulation (Cruikshank, unpublished data). IL-16 stimulation of T lymphocytes results in receptor cross-desensitization of both CCR5 and CXCR4 (Mashikian *et al.*, 1999; Van Drenth *et al.*, 2000). As a result, in the presence of IL-16, the chemokine ligands for these receptors (e.g. RANTES, MIP-1α, MIP-1β and SDF-1α) are unable to recruit T cells. The importance of this may be exemplified in inflammatory conditions that are dependent on the presence of a specific chemokine receptor such as CCR5, for example, allograft tissue rejection. Animal models have demonstrated that islet cell transplant rejection requires the presence of CCR5 (Abdi *et al.*, 2002). In humans, acute rejection and obliterative bronchiolitis are the major complications of lung transplantation and both are CD4+ T lymphocyte mediated diseases. Laan *et al.* investigated whether the concentration of IL-16 is altered in acute rejection or obliterative bronchiolitis. They measured the concentration of IL-16 in the BALF of transplant recipients at three time points and found that IL-16 is decreased in patients with acute rejection but unaltered in patients with obliterative bronchiolitis (Laan *et al.*, 2003). These results are consistent with an increase in IL-16 playing a protective role against acute lung tissue rejection.

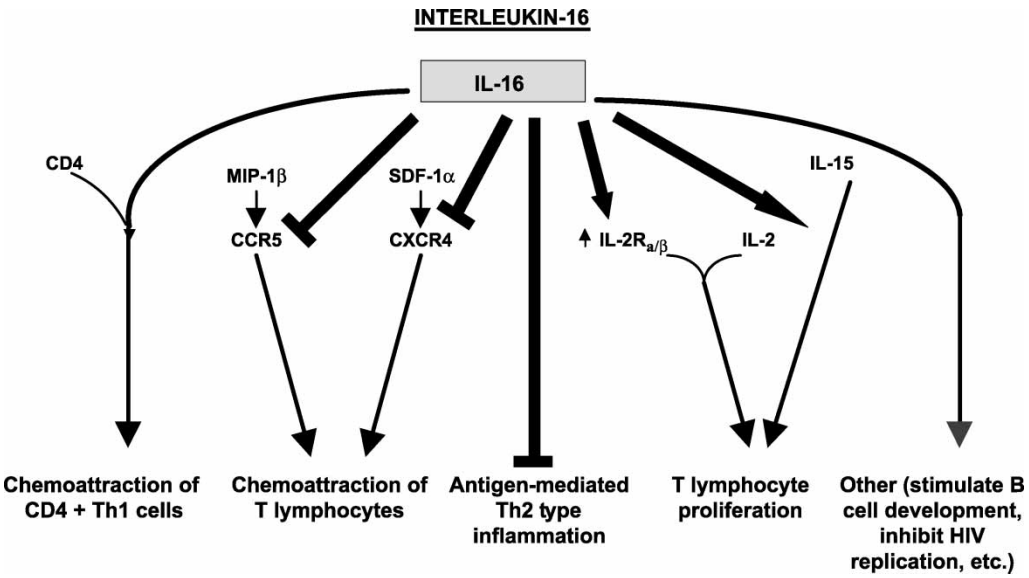

FIGURE 1 Summary of the effects of interleukin-16 on T lymphocyte-mediated inflammation, activation, and growth. Bioactive interleukin-16 (IL-16) binds to its receptor, CD4, preferentially inducing chemotaxis of CD4 + Th1 type T cells and inhibiting antigen-mediated Th2 type inflammation. The IL-16/CD4 binding interaction cross-desensitizes CCR5 and CXCR4, impairing MIP-1β and SDF-1α mediated T cell chemoattraction. In addition, IL-16 induces upregulation of IL-2Rα and IL-2Rβ, enhancing IL-2 mediated T cell proliferation. IL-15 mediated T cell proliferation is also enhanced by the presence of IL-16.

Although IL-16 has proinflammatory, pro-growth effects, these studies suggest that the inhibitory functions of IL-16 predominate in Th2-dependent antigen induced inflammation of the lung (DeBie *et al.*, 2002). Thus, through its effects on T cell recruitment and activation, IL-16 appears to induce Th1 type and inhibit Th2 type inflammatory and immune responses (Fig. 1).

While the requirement of CD4 expression for IL-16-induced migration of T lymphocytes has been well established (Cruikshank *et al.*, 1991; Ryan *et al.*, 1995; Kitchen *et al.*, 2002), the presence of an alternative receptor may exist in cells of the myloid lineage. IL-16 has been demonstrated to induce migration of CD4 negative Langerhans cells (Stoitzner *et al.*, 2001) as well as to induce cytokine production from monocytes derived from CD4 knock out mice (Mathy *et al.*, 2000). Studies are on-going to determine the nature of the alternative receptor.

IMPACT OF IL-16 ON T LYMPHOCYTE APOPTOSIS

In 1998, Zhang *et al*. exposed *in vitro* synthesized ^{35}S-labeled pro-IL-16 to lysates from *E. coli* transformed with expression vectors encoding caspase-1, caspase-3, or granzyme B. Only lysates from *E. coli* expressing caspase-3 cleaved pro-IL-16, releasing the C-terminal domain as bioactive IL-16. Caspase-3 inhibitor prevented the cleavage of pro-IL-16 and secretion of IL-16 (Zhang *et al.*, 1998b). These experiments suggest that caspase-3-mediated cleavage of pro-IL-16 is necessary for IL-16 secretion.

Caspase-3 is a potent pro-apoptotic enzyme. The findings by Zhang *et al.*, therefore, led to speculation that IL-16 may impact apoptosis. The hypothesis was advanced by demonstration that cross-linking CD4 using anti-CD4 antibodies enhances T cell apoptosis by upregulating Fas (CD95) and Fas-ligand (CD95L) expression on lymphocytes and monocytes, respectively (Oyaizu *et al.*, 1997).

Idziorek *et al*. addressed the question of whether CD4 cross-linking by IL-16 could regulate the level of spontaneous and activation-induced cell death of T cells in cultures from HIV infected patients and healthy controls. They found that addition of IL-16 to unstimulated PBMC cultures from HIV infected subjects did not modify the observed level of lymphocyte apoptosis while cultures from healthy subjects exhibited increased lymphocyte apoptosis (Idziorek *et al.*, 1998). When IL-16 was added to PBMC cultures from HIV infected subjects stimulated with anti-CD3, anti-CD95, or dexamethasone, the percentage of lymphocytes undergoing activation induced cell death was significantly reduced. This effect correlated with decreased expression of Fas on activated CD4+ T lymphocytes. In contrast, IL-16 did not alter the percentage of lymphocytes undergoing activation induced cell death in T cell cultures from healthy subjects stimulated with anti-CD3 or anti-CD95. However, like the cell cultures derived from HIV infected subjects, dexamethasone stimulation of PBMC cultures from healthy subjects resulted in diminished activation induced cell death (Idziorek *et al.*, 1998). These results are consistent with prior observations that IL-16-induced suppression of CD4+ T lymphocyte activation and proliferation are not the result of priming for activation-induced apoptosis (Cruikshank *et al.*, 1996).

Other studies have explored the impact of IL-16 on apoptosis in different leukocyte types. In CD4+ macrophages, IL-16 activates the stress-activated protein kinase (SAPK) pathway including p38 mitogen-activated protein kinase (MAPK). Despite activation of the pertinent pathways, however, IL-16 induces no detectable apoptotic cell death (Krautwald, 1998). In Jurkat CD4+ T leukemic cells, high dose IL-16 enhances apoptosis while low dose IL-16 does not (Zhang and Xu, 2002). Interestingly, although neutrophils have not been reported to express CD4 and do not respond to IL-16, the gene encoding pro-IL-16 is repressed in neutrophils undergoing apoptosis (Kobayashi *et al.*, 2003).

In summary, studies to date suggest that the impact of IL-16 on apoptosis is a complex phenomenon that likely depends on the nature of activator, the immune status of the leukocyte, and the type of leukocyte. There exists evidence that in certain circumstances IL-16 can enhance apoptosis while, in other circumstances, IL-16 inhibits apoptosis.

IL-16 INHIBITS HIV REPLICATION

Human immunodeficiency virus (HIV) requires CD4 to infect T lymphocytes. Because CD4 is the receptor for IL-16, it was hypothesized that IL-16 may act as a competitive inhibitor and reduce HIV infection. In fact, IL-16 does have anti-HIV effects, although the mechanism is different from that initially postulated.

Maciaszek *et al.*, used CD4+ lymphoid cells transiently transfected with HIV-1 LTR-reporter gene constructs to show that pre-treatment with recombinant IL-16 repressed HIV-1 promoter activity up to 60-fold (Maciaszek *et al.*, 1997). This effect required sequences contained within the HIV core enhancer, but was not simply due to the downregulation of transcription factors. The best hypothesis to date is that a repressor, induced by IL-16 / CD4 interaction, binds to the core enhancer. These findings were supported by Zhou *et al.* They transfected HIV-1 susceptible CD4+ Jurkat cells with a mammalian expression vector encoding the C-terminal 130 amino acids of IL-16 and ascertained that HIV replication was suppressed by as much as 99%. Equal amounts of HIV-1 proviral DNA were found in cells transfected with the IL-16 fragment or vector alone; however, HIV-1 transcripts were undectable (Zhou *et al.*, 1997). These findings indicate that IL-16 mediated inhibition of HIV-1 is not related to viral entry or activity of reverse transcription, but is at the level of transcription.

There exists a correlation between non-progression of AIDS with serum levels of IL-16 (Amiel *et al.*, 1999). A drop in IL-16 serum levels correlates with progression to AIDS and a rise in IL-16 levels correlates with beneficial responses to antiprotease activity (Bisset *et al.*, 1997). In addition, decreases in serum IL-16 levels are not observed in HIV-1 long-term nonprogressors (Scala *et al.*, 1997). The ability of IL-16 to prime CD4+ T lymphocytes for IL-2-induced proliferation, in combination with its anti-viral activity suggests that IL-16 may represent a feasible adjunct to IL-2 based immune reconstitution for HIV-1 infection (Center *et al.*, 2000).

PROINTERLEUKIN-16 (PRO-IL-16) IS A T LYMPHOCYTE GROWTH SUPPRESSOR

Pro-IL-16 is constitutively synthesized in T lymphocytes. It contains multiple consensus motifs that imply intracellular function. They include a protein kinase CK2 substrate site, a cdc2 kinase substrate site, a bipartite nuclear localization sequence, a putative SH3 motif, and three PDZ domains.

In human peripheral T lymphocytes, pro-IL-16 exists in both the cytoplasm and the nucleus. Following cleavage of cytoplasmic pro-IL-16 by caspase-3, the C-terminal portion autoaggregates and is secreted as bioactive IL-16. Cleavage of cytoplasmic pro-IL-16 by caspase-3 is constitutive in CD8+ T lymphocytes and induced by activation in CD4+ T lymphocytes (Zhang *et al.*, 2001b). Nuclear pro-IL-16, meanwhile, induces G0/G1 cell cycle arrest (Wu *et al.*, 1999; Wilson *et al.*, 2002b; Center *et al.*, 2003; Wilson *et al.*, 2003). In COS cells, pro-IL-16 exists in the perinuclear cytoplasm. Following cleavage by caspase 3, the C-terminal end is secreted as IL-16 and the residual N-terminal fragment translocates into the nucleus and induces G0/G1 cell cycle arrest (Wu *et al.*, 1999). This implies that nuclear pro-IL-16's ability to induce cell cycle arrest localizes to its N-terminal end.

Nuclear translocation of pro-IL-16 is tightly regulated by a CcN motif, a dual phosphorylation nuclear localization signal consisting of a protein kinase *C*K2 substrate site, *c*dc2 kinase substrate site, and bipartite nuclear localization sequence (Wilson *et al.*, 2002b). Phosphorylation of the protein kinase CK2 site enhances nuclear localization, likely by increasing the molecule's affinity for the nuclear pore complex. In contrast, phosphorylation of the cdc2 kinase substrate site impairs nuclear translocation, likely by increasing the molecule's affinity for a cytoplasmic retention factor. Once inside the nucleus, pro-IL-16 induces G0/G1 cell cycle arrest (Wu *et al.*, 1999; Wilson *et al.*, 2002b; Center *et al.*, 2003; Wilson *et al.*, 2003).

HTLV-1 Tax is a viral oncoprotein that infects T lymphocytes and causes uncontrolled cell cycle progression and growth. A binding interaction exists between pro-IL-16's first PDZ domain and HTLV-1 Tax's PDZ binding motif. Co-expression of both pro-IL-16 and HTLV-1 Tax negates the impact of either on the cell cycle (Wilson *et al.*, 2003).

The mechanism of pro-IL-16-induced cell cycle arrest is just a beginning to be elucidated. Nuclear expression of pro-IL-16 represses transcription of Skp2, a F-box protein of the Skp1-cullin-F-box (SCF) complex. As a result, the cell cycle inhibitor, p27kip1 is not targeted for degradation and cell cycle progression is impaired (Center *et al.*, 2003) (Fig. 2).

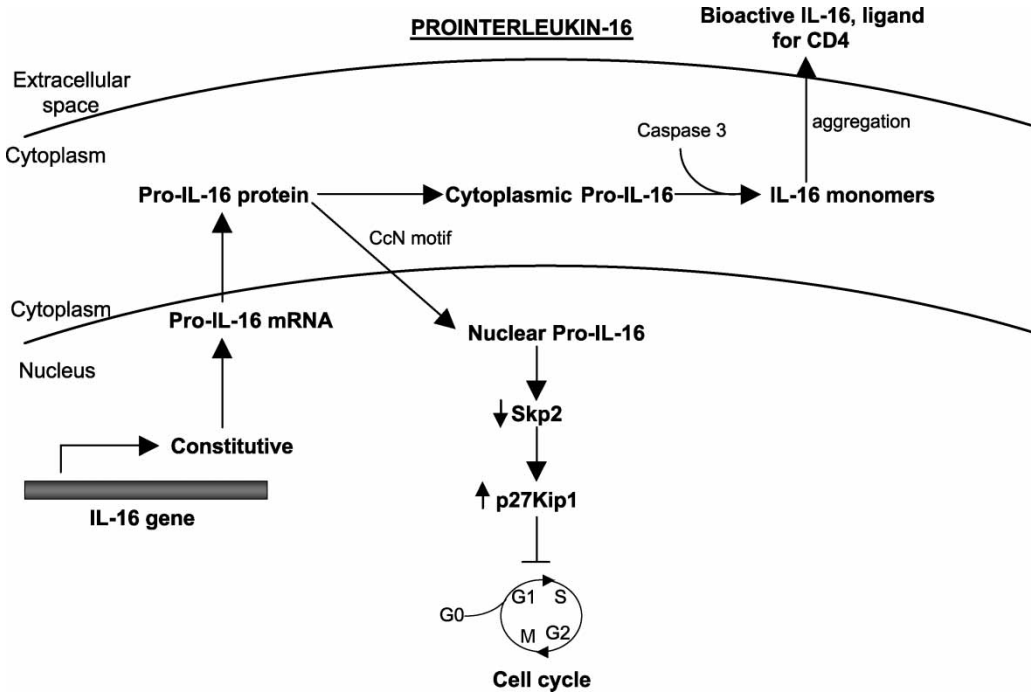

FIGURE 2 Summary of the effects of prointerleukin-16 on the T lymphocyte cell cycle. Prointerleukin-16 (pro-IL-16) protein is constitutively synthesized and localizes in both the cytoplasm and the nucleus. Cytoplasmic pro-IL-16 is cleaved by caspase 3, releasing a C-terminal fragment. The C-terminal fragment aggregates and then is secreted as bioactive interleukin-16. Nuclear translocation of pro-IL-16 is facilitated by a CcN motif, a dual-phosphorylation regulated nuclear localization signal. Nuclear pro-IL-16 transcriptionally represses Skp2, preventing Skp2 mediated degradation of the cell cycle inhibitor p27Kip1. As nuclear levels of p27Kip1 rise, cell cycle arrest results.

In an activated CD4+ human peripheral T lymphocyte, cytoplasmic pro-IL-16 is cleaved by caspase 3, liberating mature IL-16 for secretion and subsequent paracrine effects as a regulator of T cell growth. Activation causes marked transcriptional downregulation and enhanced degradation of nuclear pro-IL-16 (Feske *et al.*, 2001; Cristillo and Bierer, 2002; Zhang *et al.*, 2002), thereby permitting Skp2 transcription. Skp2 facilitates p27Kip1 degradation, resulting in cell cycle progression from G1 to S (Zhang *et al.*, 2002). Of note, downregulation of pro-IL-16 in activated T cells can be prevented by cyclosporine A and FK506 (Cristillo and Bierer, 2002).

CONCLUSION

IL-16 is an unique cytokine whereby two components of the protein, pro-IL-16 and mature IL-16, have been shown to have regulatory properties for T cell activation and growth. Mature IL-16 is secreted and directly associates with the co-receptor for the TcR, CD4, to regulate antigenic activation. Mature IL-16 is best considered as a molecule with both pro-growth and anti-growth activity in T lymphocytes. The former includes IL-16's ability to prime T lymphocytes for IL-2-dependent proliferation and in some circumstances to inhibit activation-induced apoptosis. The latter includes IL-16's ability to inhibit antigen-induced T cell activation and, in other circumstances, to promote apoptosis. There may be selectivity in the role of IL-16 during the pathogenesis of inflammation as inhibition of antigenic activation preferentially affects Th2 cells. Thus, elevated levels of IL-16 at sites of inflammation may promote a skewed Th1 response, consistent with its reported role in asthmatic inflammation. Pro-IL-16 is located both in the cytoplasm, as a pool for mature IL-16 following caspase-3 cleavage, and in the nucleus where it functions to impair degradation of p27Kip1, a key cell cycle inhibitor. Following cell activation, pro-IL-16 is lost from the nucleus, concomitant with cell cycle progression, suggesting that it plays a role in maintaining T cells in a quiescent state.

References

Abdi, R., Smith, R.N., Makhlouf, L., Najafian, N., Luster, A.D., Auchincloss, H. and Sayegh, M.H. (2002) "The role of the CC chemokine receptor 5 (CCR5) in islet allograft rejection", *Diabetes.* **51**, 2489–2495.

Amiel, C., Darcissac, E., Truong, M.J., Dewulf, J., Loyens, M., Mouton, Y., Capron, A. and Bahr, G.M. (1999) "Interleukin-16 (IL-16) inhibits human immunodeficiency virus replication in cells from infected subjects, and serum IL-16 levels drop with disease progression", *J. Infect. Dis.* **179**, 83–91.

Asadullah, K., Haeubler-Quade, A., Gellrich, S., Hanneken, S., Hansen-Hagge, T.E., Docke, W.D., Volke, H.D. and Sterry, W. (2000) "IL-15 and IL-16 overexpression in cutaneous T-cell lymphomas: stage-dependent increase in mycosis fungoides progression", *Exp. Derm.* **9**, 248.

Biddison, W.E., Kubota, R., Kawanishi, T., Taub, D.D., Cruikshank, W.W., Center, D.M., Connor, E.W., Utz, U. and Jacobson, S. (1997) "Human T cell leukemia virus type 1 (HTLV-1) specific CD8+ CTL clones from patients with HTLV-1 associated neurologic disease secrete pro-inflammatory cytokines, chemokines, and matrix metalloproteinases", *J. Immunol.* **159**, 2018–2025.

Bisset, L.R., Rothen, M., Joller-Jemelka, H.I., Dubs, R.W., Grob, P.J. and Opravil, M. (1997) "Change in circulating levels of the chemokines macrophage inflammatory proteins 1 alpha and 11 beta, RANTES, monocyte chemotactic protein 1, and interleukin 16 following treatment of severely immunodeficient HIV-infected individuals with indinavir", *AIDS* **11**, 485–491.

Blaschke, V., Reich, K., Middel, P., Letschert, M., Sachse, F., Harwix, S. and Neumann, C. (1999) "Expression of the CD4+ cell-specific chemoattractant interleukin-16 in Mycosis Fungoides", *J. Invest. Derm.* **113**, 658–663.

Cefai, D., Debre, P., Kaczorek, M., Idziorek, T., Autran, B. and Bismuth, G. (1990) "Human immunodeficiency virus-1 glycoproteins gp120 and gp160 specifically inhibit the CD3/T cell-antigen receptor phosphoinositide transduction pathway", *J. Clin. Investig.* **86**, 2117–2124.

Cefai, D., Ferrer, M., Serpente, N., Idziorek, T., Dautry-Varsat, A., Debre, P. and Bismuth, G. (1992) "Internalization of HIV glycoprotein gp120 is associated with down-modulation and membrane CD4 and $p56^{lck}$ together with impairment of T cell activation", *J. Immunol.* **149**, 285–291.

Center, D.M. and Cruikshank, W.W. (1982) "Modulation of lymphocyte migration by human lymphokines. I. Identification and characterization of chemoattractant activity for lymphocytes from mitogen-stimulated mononuclear cells", *J. Immunol.* **128**, 2563–2568.

Center, D.M., Kornfeld, H., Ryan, T.C. and Cruikshank, W.W. (2000) "Interleukin 16: implications for CD4 functions and HIV-1 progression", *Immunol. Today* **21**, 273–279.

Center, D.M., Cruikshank, W.W. and Zhang, Y. (2003) "Nuclear pro-IL-16 regulation of T Cell proliferation: $p27^{KIP1}$-dependent G_0/G_1 arrest mediated by inhibition of Skp2 transcription p27kip1", *J. Immunol.* **172**, 1654–1660.

Chirmule, N., Kalyanaraman, V.S., Oyaizu, N., Slade, H.B. and Pahwa, S. (1990) "Inhibition of functional properties of tetanus antigen-specific T cell clones by envelope glycoprotein gp120 of human immunodeficiency virus", *Blood* **75**, 152–162.

Cristillo, A.D. and Bierer, B.E. (2002) "Identification of novel targets of immunosuppressive agents by cDNA-based microarray analysis", *J. Biol. Chem.* **277**, 4465–4476.

Cruikshank, W.W. and Center, D.M. (1982) "Modulation of lymphocyte migration by human lymphokines. I. Identification and characterization of chemoattractant activity for lymphocytes from mitogen-stimulated mononuclear cells", *J Immunol.* **128**, 2569–2571.

Cruikshank, W., Berman, J., Theodore, A., Bernardo, J. and Center, D.M. (1987) "Lymphokine activation of T4+ T lymphocytes and monocytes", *J. Immunol.* **138**, 3917.

Cruikshank, W.W., Greenstein, J.L., Theodore, A.C. and Center, D.M. (1991) "Lymphocyte chemoattractant factor induces CD4-dependent intracytoplasmic signaling in lymphocytes", *J. Immunol.* **146**, 2928–2934.

Cruikshank, W.W., Long, A., Tarpy, R.E., Kornfeld, H., Carroll, M.P., Teran, L., Holgate, S.T. and Center, D.M. (1995) "Early identification of interleukin-16 (lymphocyte chemoattractant factor) and macrophage inflammatory protein 1 alpha (MIP1 alpha) in bronchoalveolar lavage fluid of antigen-challenged asthmatics", *Am. J. Respir. Cell Mol. Biol.* **13**, 738–747.

Cruikshank, W.W., Lim, K., Theodore, A.C., Cook, J., Fine, G., Weller, P.F. and Center, D.M. (1996) "IL-16 inhibition of CD3-dependent lymphocyte activation and proliferation", *J. Immunol.* **157**, 5240–5248.

DeBie, J.J., Jonker, E.H., Henricks, P.A., Hoevenaars, J., Little, F.F., Cruikshank, W.W., Nijkamp, F.P. and Van Oosterhout, A.J. (2002) "Exogenous interleukin-16 inhibits inhibits antigen-induced airway hyperreactivity, eosinophilia, and Th2-type cytokine production in mice", *Clin. Exp. Allergy* **32**, 1651–1658.

Feske, S., Giltnane, J., Dometsch, R., Staudt, L.M. and Rao, A. (2001) "Gene regulation mediated by calcium signals in T lymphocytes", *Nat. Immunol.* **2**, 316–324.

Hessel, E.M., Cruikshank, W.W., Van Ark, I., de Bie, J.J., Van Esch, B. and Hofman, G. (1998) "Involvement of IL-16 in the induction of airway hyperresponsiveness and upregulation of IgE in a murine model of allergic asthma", *J. Immunol.* **160**, 2998–3005.

Idziorek, T., Khalife, J., Billaut-Mulot, O., Hermann, E., Aumercier, M., Mouton, Y., Capron, A. and Bahr, G.M. (1998) "Recombinant human IL-16 inhibits HIV replication and protects against activation-induced cell death (AICD)", *Clin. Exp. Immunol.* **112**, 84–91.

Kaser, A., Dunzendorfer, S., Offner, F.A., Ryan, T., Schwabegger, A., Cruikshank, W.W., Wiedermann, C.J. and Tilg, H. (1999) "A role for IL-16 in the cross-talk between dendritic cells and T cells", *J. Immunol.* **162**, 3232–3238.

Keates, A.C., Castagliuolo, I., Cruickshank, W.W., Qiu, B., Arseneau, K.O., Brazer, W. and Kelly, C.P. (2000) "Interleukin 16 is up-regulated in Crohn's disease and participates in TNBS colitis in mice", *Gastroenterology* **119**, 972–982.

Kitchen, S.G., LaForge, S., Patel, V.P., Kitchen, C.M., Miceli, M.C. and Zack, J.A. (2002) "Activation of CD8 T cells induces expression of CD4, which functions as a chemotactic receptor", *Blood* **99**, 207–212.

Kobayashi, S.D., Voyich, J.M., Braughton, K.R. and DeLeo, F.R. (2003) "Downregulation of proinflammatory capacity during apoptosis in human polymorphonuclear leukocytes", *J. Immunol.* **170**, 3357–3368.

Krautwald, S. (1998) "IL-16 activates the SAPK signaling pathway in CD4+ macrophages", *J. Immunol.* **160**, 5874–5879.

Kurschner, C. and Yuzaki, M. (1999) "Neuronal IL-16 (NIL-16): a dual function PDZ domain protein", *J. Neurosci.* **19**, 7770–7780.

Laan, M., Linden, A. and Riise, G.C. (2003) "IL-16 in the airways of lung allograft recipients with acute rejection or obliterative bronchiolitis", *Clin. Exp. Immunol.* **133**, 290–296.

Laberge, S., Durham, S.R., Ghaffar, O., Rak, S., Center, D.M., Jacobson, M. and Hamid, Q. (1997) "Expression of IL-16 in allergen-induced late-phase nasal responses and relation to topical glucocorticosteroid treatment", *J. Allergy Clin. Immunol.* **100**, 569–574.

Laberge, S., Ernst, P., Ghaffar, O., Cruikshank, W.W., Kornfeld, H., Center, D.M. and Hamid, Q. (1997) "Increased expression of interleukin-16 in bronchial mucosa of subjects with atopic asthma", *Am. J. Respir. Cell Mol. Biol.* **17**, 193–202.

Lim, K.G., Wan, H.C., Bozza, P.T., Resnick, M.B., Wong, D.T., Cruikshank, W.W., Kornfeld, H., Center, D.M. and Weller, P.F. (1996) "Human eosinophils elaborate the lymphocyte chemoattractants. IL-16 (lymphocyte chemoattractant factor) and RANTES", *J. Immunol.* **156**, 2566–2570.

Little, F.F., Cruikshank, W.W. and Center, D.M. (2001) "IL-9 stimulates release of chemotactic factors from human bronchial epithelial cells", *Am. J. Respir. Cell Mol. Biol.* **25**, 347–352.

Little, F.F., de Bie, J., van Oosterhous, A., Kornfeld, H., Center, D.M. and Cruikshank, W.W. (2003) "Immunomodulatory effect of interleukin-16 on allergic airway inflammation [Review]", *Chest* **123**, 431S–432S.

Lombard, C., McKallip, R.J., Hylemon, P.B., Nagarkatti, P.S. and Nagarkatti, M. (2003) "Fas ligand-dependent and independent mechanisms of toxicity induced by T cell lymphomas in lymphoid organs and in the liver", *Clin. Immunol.* **109**, 144–153.

Lynch, E.A., Little, F.F., Wilson, K.C., Center, D.M. and Cruikshank, W.W. (2003) "Immunomodulatory cytokines in asthmatic inflammation [Review]", *Cytokine Growth Factor Rev.* **14**, 489–502.

Lynch, E.A., Heijens, C.A., Horst, N.F., Center, D.M. and Cruikshank, W.W. (2003) "Cutting edge: IL-16 / CD4 preferentially induces Th1 cell migration: requirement of CCR5", *J. Immunol.* **171**, 4965–4968.

Maciaszek, J.W., Parada, N.A., Cruikshank, W.W., Center, D.M., Kornfeld, H. and Viglianti, G.A. (1997) "IL-16 represses HIV-1 promoter activity", *J. Immunol.* **158**, 5–8.

Mashikian, M.V., Ryan, T.C., Seman, A., Brazer, W., Center, D.M. and Cruikshank, W.W. (1999) "Reciprocal desensitization of CCR5 and CD4 is mediated by IL-16 and macrophage inflammatory protein-1 beta, respectively", *J. Immunol.* **163**, 3123–3130.

Mathy, N.L., Bannert, N., Norley, S.G. and Kurth, R. (2000) "Cutting edge: CD4 is not required for the functional activity of IL-16", *J. Immunol.* **164**, 4429–4432.

Mi, Q.S., Meagher, C. and Delovitch, T.L. (2003) "CD1d-restricted NKT regulatory cells: functional genomic analyses provide new insights into the mechanisms of protection against Type 1 diabetes", *Novartis Found Symp.* **252**, 146–160.

Oyaizu, N., Adachi, Y., Hashimoto, F., McCloskey, T.W., Hosaka, N., Kayagaki, N., Yagita, H. and Pahwa, S. (1997) "Monocytes express Fas ligand upon CD4 cross-linking and induce CD4+ T cell apoptosis: a possible mechanism of bystander cell death in HIV infection", *J. Immunol.* **158**, 2456–2463.

Parada, N.A., Center, D.M., Kornfeld, H., Rodriquez, W.L., Cook, J., Vallen, M. and Cruikshank, W.W. (1998) "Synergistic activation of CD4+ T cells by IL-16 and IL-2", *J. Immunol.* **160**, 2115–2120.

Pinsonneault, S., El Bassam, S., Mazer, B., Cruikshank, W.W. and Laberge, S. (2001) "IL-16 inhibits IL-5 production by antigen-stimulated T cells in atopic subjects", *J. Allergy Clin. Immunol.* **107**, 477–481.

Rumsaeng, V., Cruikshank, W.W., Foster, B., Prussin, C., Kirshenbaum, A.S., Davis, T.A., Kornfeld, H., Center, D.M. and Metcalfe, D.D. (1997) "Human mast cells produce the CD4+ T lymphocyte chemoattractant factor, IL-16", *J. Immunol.* **159**, 2904–2910.

Ryan, T.C., Cruikshank, W.W., Kornfeld, H., Collins, T.L. and Center, D.M. (1995) "The CD4-associated tyrosine kinase p56lck is required for lymphocyte chemoattractant factor-induced T lymphocyte migration", *J. Biol. Chem.* **270**, 17081–17086.

Scala, E., D'Offizi, G., Rosso, R., Turriziani, O., Ferrara, R., Mazzone, A.M., Antonelli, G., Aiuti, F. and Paganelli, R. (1997) "C-C chemokines, IL-16, and soluble antiviral factor activity are increased in cloned T cells from subjects with long-term nonprogressive HIV infection", *J. Immunol.* **158**, 4485–4492.

Sciaky, D., Brazer, W., Center, D.M., Cruikshank, W.W. and Smith, T.J. (2001) "Cultured human fibroblasts express constitutive IL-16 mRNA: cytokine induction of active IL-16 protein synthesis through a caspase-3-dependent mechanism", *J. Immunol.* **164**, 3806–3814.

Sharma, V., Sparks, J.L. and Vail, J.D. (2000) "Human B-cell lines constitutively express and secrete interleukin-16", *Immunology* **99**, 266–271.

Stoitzner, P., Ratzinger, G., Koch, F., Janke, K., Scholler, T., Kaser, A., Tilg, H., Cruikshank, W.W., Fritsch, P. and Romaini, N. (2001) "Interleukin-16 supports the migration of Langerhans cells, partly in a CD4-independent way", *J. Invest. Dermatol.* **116**, 641–649.

Theodore, A.C., Center, D.M., Nicoll, J., Fine, G., Kornfeld, H. and Cruikshank, W.W. (1996) "CD4+ ligand IL-16 inhibits the mixed lymphocyte reaction", *J. Immunol.* **157**, 1958–1964.

Van Drenth, C., Jenkins, A., Ledwich, L., Ryan, T.C., Mashikian, M.V., Brazer, W., Center, D.M. and Cruikshank, W.W. (2000) "Desensitization of CXC chemokine receptor 4, mediated by IL-16/CD4, is independent of p56lck enzymatic activity", *J. Immunol.* **165**, 6356–6363.

Wilson, K.C., Cruikshank, W.W., Center, D.M. and Zhang, Y. (2002) "The N-terminal domain of prointerleukin-16 contains a functional CcN motif", *Biochemistry* **41**, 14306–14312.

Wilson, K.C., Center, D.M. and Zhang, Y. (2003) "Binding of HTLV-1 Tax oncoprotein to the precursor of Interleukin-16, a Tcell PDZ Domain containing protein", *Virology* **306**, 60–67.

Wu, D.H., Zhang, Y., Parada, N.A., Kornfeld, H., Nicoll, J., Center, D.M. and Cruikshank, W.W. (1999) "Processing and release of IL-16 from CD4+ but not CD8+ T cells in activation dependent", *J. Immunol.* **162**, 1287–1293.

Yoshimoto, T., Wang, C.R., Yoneto, T., Matsuzawa, A., Cruikshank, W.W. and Nariuchi, H. (2000) "Role of IL-16 in delayed-type hypersensitivity reaction", *Blood* **95**, 2869–2874.

Zhang, X.M. and Xu, Y.H. (2002) "The associated regulators and signal pathway in IL-16 / CD4 mediated growth regulation in Jurkat cells", *Cell Res.* **12**, 363–372.

Zhang, Y., Center, D.M., Wu, M.H., Cruikshank, W.W., Yuan, J., Andrews, D.W. and Kornfeld, H. (1998) "Processing and activation of prointerleukin-16 by caspase-3", *J. Biol. Chem.* **273**, 1144–1149.

Zhang, Y., Kornfeld, H., Cruikshank, W.W., Kim, S., Reardon, C.C. and Center, D.M. (2001) "Nuclear translocation of the N-terminal prodomain of interleukin-16", *J. Biol. Chem.* **276**, 1299–1303.

Zhang, X., Chen, Z., Huang, H., Gordon, J.R. and Xiang, J. (2002) "DNA microarray analysis of the gene expression profiles of naïve versus activated tumor-specific T cells", *Life Sci.* **71**, 3005–3017.

Zhou, P., Goldstein, S., Devadas, K., Tewari, D. and Notkins, A.L. (1997) "Human CD4+ cells transfected with IL-16 cDNA are resistant to HIV-1 infection: inhibition of mRNA expresstion", *Nat. Med.* **3**, 659–664.