Scientific Report 2005-2006

Institute for Research in Biomedicine, Istituto di Ricerca in Biomedicina

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Giorgio Noseda, President of the Foundation Council

When we formed the Foundation for the Institute for Research in Biomedicine in 1997 our objective was to create the ideal conditions for the establishment of a world-class research institute in Ticino. The overwhelming success of the IRB in its first seven years attests to our having reached that ambitious goal. Scientific and academic productivity of the IRB are well documented in 187 publications and 22 PhD thesis granted to our students. The growth of the annual budget from 3.8 million Fr. in 2000 to more than **11.3 million Fr**. in 2006 is due to both the trust demonstrated by our public and private sponsors (approx. **60%** of the total) as well as by the increasing ability of our researchers to attract competitive grants.

Preparing the ground for success is not the same as managing success however. The year 2006 saw an important strategic transition begin for the Foundation Council. No longer the"new kid on the block", the IRB now successfully competes head to head with the leading research institutions worldwide. Remaining competitive at this level the Foundation and the Institute must offer ideal conditions, which include solidity, predictability, management excellence, as well as an increased involvement in the academic landscape of Switzerland to provide recognition of the excellence of our researchers.

In 2006 we prepared our renewed requests to the Cantonal and Federal governments for increased future funding. We solidified our connections with Swiss Universities and in particular opened discussions with the Swiss polytechnic institutes. In December of 2006 the IRB, together with the EPFL, the CHUV (University of Lausanne) and the Ludwig Institute for Cancer Research formed the Swiss Institute for Vaccine Research. These four institutes will pursue a coordinated program for research on vaccines.

Funds provided by the Bill and Melinda Gates Foundation to individual researchers will be matched by the Confederation to accelerate this program.

Since 2000 the institute has grown to **75** people; **9** group leaders, **22** researchers, **30** students, **9** technicians and an administrative staff of **7**. This growth has put intense pressure on our lab space and infrastructure. To ease this pressure, in June of 2006, we expanded our facilities through the transformation of a local building into a state of the art laboratory and animal facility.

Joining the IRB as tenants in this new building will be Humabs, a young U.S. based biotechnology company that has recently opened a swiss office in Ticino. Humabs has licensed IRB technology, developed by Prof. Antonio Lanzavecchia, for the production of monoclonal antibodies. The Foundation believes strongly in the importance of academic and industrial alliances to foster important and effective research programs without compromising independence.

While the Foundation takes stock of the past six years we also look forward with renewed enthusiasm to the challenges that lie ahead. We will transition ourselves be in the best position possible to achieve our ambitions and the ambitions of our scientists.

Giorgio Noseda, MD

Antonio Lanzavecchia, Director

Introduction

This report provides an overview of last two years of activity at the Institute for Research in Biomedicine (IRB), and gives insight into ongoing research projects.

Three research groups have been recruited during this period. Markus Manz, after completing the qualification in hematology and oncology at the University of Tubingen, has expanded his research program on stem cell biology, hematopoietic differentiation, and blood cell maligancies. Jeremy Luban, on sabbatical from the Columbia University since 2005, has joined the institute as a group leader in August 2006. His research on the innate mechanisms of resistance to HIV-1 will strengthen the IRB program in this important field. In addition, his laboratory is undertaking the challenging task of tracking viral transport inside infected cells using a novel microscopy approach. Silvia Monticelli, recruited from Harvard University in 2007, works on micro-RNA, an emerging field with impact in development and oncology. Scientists that have left the IRB in this period have found positions in leading institutions in Switzerland, Germany, Italy, USA, Canada, UK, Japan and Singapore.

The IRB continues to play an important role in education by training graduate students through collaborations with Swiss Universities, in particular Basel, Bern, Fribourg, Lausanne and Zurich and participates in an international PhD program with the Vita-Salute San Raffaele University in Milan, Italy. The students furthermore benefit from an intensive lecture series held by renowned scientist from all over the world, organized with the generous support of the Gustav & Ruth Jacob Foundation. In turn, the IRB greatly profits from the curiosity and ambitious work of our young scientists. At present 30 graduate students are enrolled at the IRB and since beginging in 2002, 22 students have successfully completed their training.

Research at the IRB has increasingly focused on the study of host defense mechanisms in the human system. IRB researchers have developed coherent programs that have the potential to be translated into novel therapies. Manz and colleagues have shown that mice reconstituted with a human hematolymphoid system can be successfully infected with viruses that target human cells but normally do not infect animals. This work provides, for the first time, a small animal model for long-lasting HIV infection and is supported by the Bill & Melinda Gates Foundation Grand Challenges in Global Health program. The need to advance the study of the human immune system is illustrated by the finding by Sallusto and colleagues that the development of a particular type of inflammatory effector cells, called Th17, is differentially regulated in humans as compared to mice. A very dynamic program stems from the development of a proprietary method to clone human memory B cells which has been licensed to an american startup company (Humabs LLC) that has established its laboratories in Bellinzona. Using this proprietary method broadly neutralizing antibodies have been isolated against viruses such as SARS, Dengue, H5N1, Cytomegalovirus and HIV-1. These antibodies can be used not only to confer immediate protection by passive administration to virus-exposed or infected individuals, but also as tools to identify the antigens that elicit neutralizing antibodies, a process that has been defined as "analytic vaccinology". Another example of how basic research can open new therapeutic avenues comes from Molinari's laboratory where they have shown in a model system that an antibody can inhibit the formation of Alzheimer lesions *in vivo*.

The translational programs above stem directly from strong basic research, which remains the mission of the IRB. The scientific impact of this research rests on the **187** publications that are listed with average impact factor of 11.9 at the end of this report and which deal with molecular aspects of protein trafficking and quality control, cell signaling migration and differentiation, and with immune effector mechanisms in normal and pathological conditions.

IRB scientists have established an effective network of collaborations with leading institutions in Europe, America and Asia.

The IRB is a Founding member of the Swiss Vaccine Research Institute (SVRI) together with the CHUV, the EPFL and the Ludwig Institute in Lausanne. The aim of the SVRI is to boost vaccine research and development via creation of common platforms and recruitment of young talents. Effective collaborations have been established with the sister institute IOSI in the field of transcriptional profiling and in the study of the effect of irradiation on adoptive immunotherapy. Markus Manz has taken clinical responsibilities at the IOSI, a move that is expected to facilitate translational research in the field of haemato-oncology.

The urgent need to expand laboratory space, core facilities, and the animal house has been partially met by the rapid refurbishment of a new building called IRBis located a few hundred meters from the main site, and by establishing a cutting edge imaging facility, generously supported by private donations and the Confederation. We are grateful to the City of Bellinzona for hosting us in these two buildings and for sharing our vision of the future.

After seven years of activity I am confident to say that the IRB has fulfilled the initial expectations and has become an internationally visible centre for basic and translational research. This fact is witnessed by the success in obtaining grants not only from the Swiss National Science Foundation and the European Union, but also from foreign agencies among which are the Bill & Melinda Gates Foundation, the Wellcome Trust, and the US National Institute of Health.

The Institute is especially fortunate in receiving core funding from its main sponsors, the Helmut Horten Foundation, the Cantone Ticino and the Swiss Confederation. Our gratitude also goes to the many individuals who support us through donations and fellowships. We believe that the progress and achievements of the Institute will reward their dedication to the advancement of science.

Antonio Lanzavecchia, MD

Cultivating Discovery

The IRB seeks to provide the ideal conditions for the creation of new knowledge.

IRB scientists participate in the virtuous cycle of discovery by asking important questions, learning what is known, building new knowledge and sharing their discoveries.

Advancing Discovery

Teaching

Discovery

The cowbell is rung to call students to a seminar

The IRB is a dynamic teaching institute with 30 full time graduate students from Swiss and international universities.

Focusing
Discovery

IRB research programs explore how the human body defends itself.

The cafeteria is in the heart of the building, an open space where students and scientists gather and exchange ideas.

SHAPING PROTEINS

Maurizio Molinari earned his PhD in Biochemistry at the ETH-Zurich in 1995. From 1996-1997 he was a post-doc in the laboratory of Cesare Montecucco at the Dept. of Biomedicine, University of Padua, Italy and subsequently in the laboratory of Ari Helenius at the ETH-Zurich (1998-2000). Since October 2000 he is group leader at the IRB in Bellinzona.

The studies performed by Molinari's group at the IRB have made a significant contribution to the knowledge of the mechanisms devised by cells for the production of functional polypeptides and for efficient disposal of folding-defective proteins.

The knowledge acquired on the mechanisms of protein production and transport along the secretory line of mammalian cells allowed the group to set up a novel approach based on intracellular expression of specific single chain antibodies, that proved very efficient in reducing the in vivo production of amyloid -Beta (Aβ), a toxic peptide that deposits in the human brain and elicits neurodegenerative processes associated with Alzheimer's disease.

Dr. Molinari has received the Science Award 2002 from the Foundation for study of neurodegenerative diseases, the Kiwanis Club Award 2002 for Medical Science, the Friedrich-Miescher Award 2006 and the Research Award Aetas 2007.

Protein Folding and Quality Control

Cystic fibrosis, Alzheimer's disease, Parkinson's disease, Hungtington disease, Creutzfeld-Jakob's disease, diabetes mellitus. This is a very short selection of the several hundreds of "human diseases" with profoundly different traits but common aethiology: "protein misfolding". Several of these diseases are hereditary and most of them are rare, affecting less than 1 in 2000 individuals. For some of them, as an example for all neurodegenerative syndromes affecting old people, a sharp increase in frequency is expected in the next decades due to extension of the human life span.

Proteins are fundamental components of all living cells and include many substances, such as enzymes, hormones, and antibodies, that are necessary for the proper functioning of an organism. They are fabricated in a specialized organelle present in all our cells and named the endoplasmic reticulum.

To be functional, proteins must acquire a specific structure in the endoplasmic reticulum in a series of tightly regulated processes defined as "protein folding". Failure to do so, will result in the destruction of the defective protein and in the loss of its activity. The cell and the organism will eventually suffer as a consequence of the absence of this specific protein's function and this elicits a disease state.

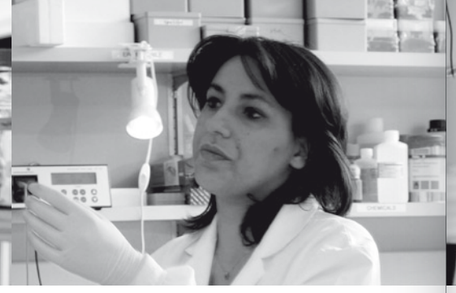
The aim of our work is to understand how our cells fabricate the proteins. How correct protein folding is ensured, what happens if protein folding is not possible, how misfolded proteins are destroyed. We are convinced that a detailed knowledge of the processes that regulate protein production in our cells will allow intervention to alleviate symptoms, to delay disease onset and even to arrest and revert disease progression for all human pathologies caused by defects in acquisition of the functional protein shape.

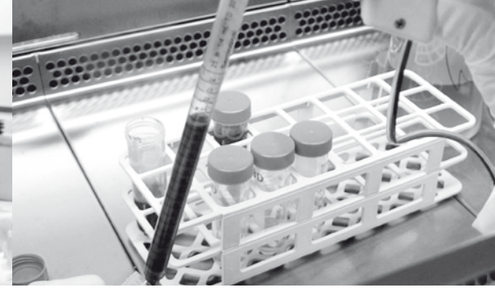
Laboratory

Group Leader: Maurizio Molinari, *PhD, 2000.*

Members: Verena Calanca, *Technician, 2000* • Carmela Galli-Molinari, *2000* • Tatiana Soldà, *Technician, 2004* • Omar Vanoni, *PhD student, 2004* • Tito Calì, *PhD student, 2006* • Silvia Olivari, *PhD Student, 2006-2007* • Siro Bianchi, *Technician, 2007* • Riccardo Bernasconi, *PhD student, 2007.*





Research Projects

ER-associated degradation of folding-defective glycoproteins.

Silvia Olivari, Tito Calì, Carmela Galli, and Maurizio Molinari.

Newly synthesized glycoproteins are subjected to a quality control necessary for the maintenance of the ER homeostasis. Persistent association with ER-resident molecular chaperones prevents exit of misfolded or incompletely folded polypeptides from the ER and their forward transport along the secretory line. Folding-defective proteins carrying N-linked glycans undergo futile folding attempts in the calnexin chaperone system (Fig. 2). Futile folding attempts are eventually interrupted, folding-incompetent polypeptides are extracted from the folding environment, are transported across the ER membrane into the cytosol and are degraded by the 26S proteasome in a series of tightly regulated processes collec-tively named ER-associated degradation (ERAD). Overexpresison of EDEM1 (ER-Degradation Enhancing α-Mannosidase like protein) accelerates extraction of folding-defective glycoproteins from the calnexin cycle thereby accelerating their disposal (Molinari et al. 2003).

We determined that EDEM1 enhances ERAD in two different and independent ways: i) EDEM1 regulates removal of α1,2-bonded mannose residues (Fig. 1) from N-glycans displayed on misfolded proteins. ii) EDEM1 prevents aggregation of terminally misfolded proteins released from calnexin substrate. Our findings suggest, against current dogmas, that EDEM1 is an enzymatically active member of the family 47 glycosyl hydrolases that can also operate as molecular chaperone to inhibit protein aggregation. We identified and characterized two novel mammalian paralogs of EDEM1 and named them EDEM2 and EDEM3. EDEM1, EDEM2 and EDEM3 are stress-inducible proteins responding to the Ire1/Xbp1 unfolded protein response pathway.

• *Olivari S. et al. / J Biol Chem 2005; 280:2424-2428* • *Olivari S. et al. / Biochem Biophys Res Commun 2006; 349:1278-1284* • *Olivari S. and Molinari M. / FEBS Lett 2007; 581, 3658-3664*

ER-associated degradation and macroautophagy.

Tito Calì, Silvia Olivari, Carmela Galli, and Maurizio Molinari.

(Macro)autophagy is a unique intracellular process in which membrane-bound compartments engulf organelles and macromolecules and deliver them to lysosomes for destruction. Recent experiments performed in cell lines lacking macroautophagy upon deletion of ATG5 (a gene product required to initiate the autophagic process) eventually showed accumulation of misfolded proteins in the ER lumen. This led to a model proposing that autophagy contributes to disposal of folding-defective polypeptides synthesized in the ER. We could only partially confirm these published results.

More precisely, we found that deletion of autophagy inhibits protein degradation from the ER lumen only when cells are exposed to acute conditions of stress such as amino acids and serum deprivation. We found that when cells are grown under normal nutrient supply, inactivation of autophagy actually enhances protein degradation from the ER. This finding led to several unanticipated results. i) autophagy regulates the turnover and the intracellular content of select ER-resident chaperones (e.g. EDEM1 and OS9, two glycanases/lectins regulating glycoprotein disposal from the ER). Cells lacking autophagy have higher content of these factors and show hyper-activity of the ERAD machinery. ii) Folding programs are prematurely interrupted in cells with hyper-active ERAD machinery. This highlights the kinetic competition ongoing in the ER lumen where nascent polypeptide chains are exposed to a machinery aiming at folding them and to a machinery aiming at degrading them. iii) the ER can be separated in sub-compartments (that we named transitional and peripheral ER) with different chaperone content. Chaperones with short half-life (e.g. EDEM1 and OS9) are enriched in the peripheral ER a sub-region accessible to the autophagic machinery. Chaperones with long half-life (the vast majority of the ER resident proteins) are found in the transitional ER. Their half-life and intracellular level is unaffected by changes in the autophagic activity.

Protein aggregation as an intermediate step in ERAD.

Silvia Olivari and Maurizio Molinari.

Proteins that are unable to fold correctly in the ER are dislocated into the cytosol and degraded by the proteasome. The series of events eventually leading to ERAD may vary depending on the characteristic of the ERAD substrate and of the cell line. Previous work has shown that at the end of a lag phase consisting in unproductive folding attempts in the calnexin cycle, ERAD candidates are released from calnexin and enter, transiently, in BiP- and PDI-associated disulfide-bonded complexes before dislocation into the cytosol (Molinari et al. 2002). We also found that the intralumenal level of EDEM determines kinetics of ERAD by regulating glycoprotein release from the calnexin cycle (Molinari et al. 2003). Studies on the mechanisms by which EDEM1 overexpression facilitates disposal of folding-defective glycoproteins led to establish a dual mode of action of this member of the glycosyl hydrolase 47 family comprising several ER- and Golgi-resident α1,2-mannosidases. EDEM1 operates by accelerating the extensive de-mannosylation that promotes substrate extraction from the calnexin chaperone system. It also can act as a molecular chaperone that inhibits aggregation of terminally misfolded proteins released from calnexin.

• *Olivari S. et al. / Biochem Biophys Res Commun 2006; 349:1278-1284.*

The role of UDP-glucose: glycoprotein glucosyl-transferase (UGT) in glycoprotein quality control.

Tatiana Soldà, Carmela Galli, Omar Vanoni and Maurizio Molinari.

Newly synthesized polypeptides are co-translationally N-glycosylated by addition of pre-assembled, triglucosylated oligosaccharides on asparagine residues in Asn-X-Ser / Thr sequons (*Fig.1*). Termi-nal glucoses of N-glycans are rapidly trimmed by sequential action of glucosidases I and II. The product of these trimming reactions is a mono-glucosylated, protein-bound N-glycan that mediates association of the nascent polypeptide with the ER-lectins calnexin and calreticulin. Association with the two ER lectins exposes nascent chains to the glycoprotein-specific oxidoreductase ERp57 that catalyzes rate-limi-ting reactions of the folding process, namely the formation and isomerization of intra- and intermolecular disulfide bonds. Cleavage of the last glucose residue releases the glycopolypeptide from calnexin or calreticulin and exposes it to a structural check operated by the UGT. This enzyme specifically adds back one glucose on N-glycan of non-native proteins that require longer retention in the calnexin cycle. ER quality control ensures that only native polypeptides leave the ER to be transported to their final destination. We are investigating consequences of depletion of the ER folding sensor UGT on protein biosynthesis with the aim of better understanding the molecular mechanisms regulating protein quality control in the mammalian ER.

So far, we established that tight quality control is maintained by two distinct chaperone systems retaining folding defective polypeptides by acting sequentially. In a first phase of retention-based ER quality control, immature glycoproteins are retained in the ER folding environment due to persistent re-glucosylation operated by the folding sensor UGT1 that prevents substrate release from the calnexin chaperone system. In a second phase, when terminally misfolded proteins are eventually released from the calnexin chaperone system following extensive de-mannosylation of their N-glycans, their forward transport is inhibited by the BiP chaperone system. Altogether, these quality control mechanisms efficiently prevent release into the secretory pathway of non-native polypeptides.

• Molinari M. et al. / *Mol Cell 2005;20:503-512*
• Soldà T. et al./ *Mol Cell 2007; 27, 238-249*

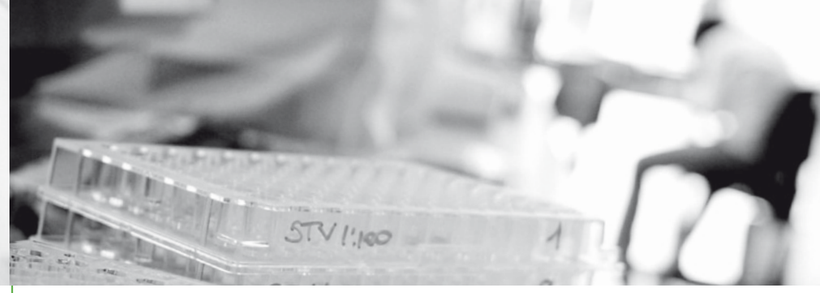
The role of the ER-resident oxidoreductase ERp57 in oxidative glycoprotein folding.

Tatiana Soldà and Maurizio Molinari.

The ER contains several molecular chaperones and folding factors that faci-litate the folding and the assembly of newly synthesized polypeptides. The two lectin chaperones calnexin and calreticulin are associated with ERp57, a lumenal member of the protein disulfide isomerase (PDI) superfamily. ERp57 specifically promotes the oxidative folding of newly synthesized glycoproteins.

The aim of this project is to determine consequences of ERp57 down-regulation and deletion on glycoprotein folding and to analyze if other ER resident oxidoreductases can replace ERp57. Cells showing substantial down-regulation of ERp57 have been obtained by RNA interference, upon intracellular expression of an ERp57 specific double-strand RNA formed by the sense and the antisense oligonucleotides connected by a short loop. ERp57 deletion is embryonic lethal for mice. To generate a ERp57-deficient cell line, fibroblasts were first prepared from ERp57$^{flox/flox}$ mice, which express wild-type levels of ERp57 protein.
A cloned line immortalised with SV40 large T antigen was then transfected with a plasmid coding for cre recombinase to obtain ERp57 knockout fibroblasts. Analysis of biosynthesis of select model substrates revealed that only influenza virus hemagglutinin (amongst the substrates tested) obligatorily requires ERp57



intervention for productive folding. More specifically, ERp57-deletion prevented the post-translational disul-fide-bond re-shuffling required to acquire a transport-competent architecture that fulfils ER quality control standards. For several other model glycoproteins, oxidative folding successfully progressed in cells lacking ERp57. We identified the functional paralog ERp72 as a surrogate oxidoreductase assisting glycoprotein maturation in cells lacking ERp57.

• Soldà T. et al. / *J Biol Chem 2006; 281:6219-6226*

Identification and characterization of novel ER-resident lectins that participate in ERAD.

Riccardo Bernasconi, Tatiana Soldà and Maurizio Molinari.

Several pathways that regulate folding, quality control and degradation of proteins synthesized in the ER are already present, in simpler form, in the budding yeast Saccharomyces cerevisiae. Recently, the ER-resident protein Yos9p has been shown to regulate disposal from the yeast ER of aberrant glycopolypeptides displaying folding defects in their luminal ectodomain. A human ortholog of Yos9p has not yet been identified. Erlectin and OS9 are possible candidates. Erlectin has recently been identified as an ER-resident lectin assisting export from the ER of select secretory proteins. Os9 has been reported to be associated with the cytosolic face of the ER membrane, a topology that would be incompatible with a role of this protein as a lectin in the ER lumen. Our work in progress already led to establish that the Os9 topology is wrong, as described in the literature. Os9 is synthesized in two splice variants in the lumen of the ER. We also established that Os9 variants are stress-regulated proteins whose transcriptional induction depends on activation of the Ire1/Xbp1 UPR pathway. This is a hallmark of proteins involved in ERAD regulation (e.g. EDEM1, EDEM2, EDEM3 are all induced upon activation of Ire1/Xbp1). Up-regulation and down-regulation of Os9 expression specifically retard disposal of soluble glycoproteins from the ER. We are trying to explain this interesting conundrum.

Involvement of several ER-resident proteins in ERAD: studies with knockout cells.

Carmela Galli, Verena Calanca, Tatiana Soldà and Maurizio Molinari.

We started several international collaborations to establish in Bellinzona a collection of cell lines lacking select ER-resident factors.

The aim is to use our expertise in this field to understand the involvement of these ER-resident factors in protein biogenesis and quality control, the consequences of their deletion and the responses activated by cells to compensate their absence.

Amongst the available knockout cell lines there are mouse embryonic fibroblasts lacking calnexin, calreticulin, ERp57, UGT1, p58, synoviolin, ATG5, Xbp1 and with reduced expression of EDEM, Os9, cyclophillin B, BiP,…

Novel approaches to inhibit production of the toxic Aβ peptide.

Omar Vanoni, Siro Bianchi and Maurizio Molinari.

The human amyloid precursor protein (APP) is one of the model-substrates used in our lab to investigate protein folding and quality control. Availability of a very specific monoclonal antibody used for immunoisolation of APP from cell lysates led us to the idea of setting-up an approach to inhibit generation of the toxic amyloid-beta (Aβ), a metabolite of APP cleavage operated by the cellular beta- and gamma-secretases. We mapped the monoclonal antibody epitope in the human APP molecule to find that it was located between residues 3 and 6 of the Aβ peptide. We prepared a vector for expression in mammalian cells of a single chain antibody (intrabody) formed by the variable heavy and light chain regions of the original monoclonal antibody covalently associated through a 15 aminoacids linker. We hypothesized that expression of this intrabody in the ER (and of a second variant of the same intrabody carrying an ER retention sequence) would result in association of the intrabody with newly synthesized APP, shielding of the APP's beta-secretase cleavage site adjacent to the intrabody epitope, and consequent inhibition of Aβ production. The experimental data confirmed our thoughts. One of our intrabodies associated with APP in the ER and followed the APP molecule throughout the secretory line strongly inhibiting beta-secretase-operated cleavage. The intrabody displaying the ER retention sequence also associated, and retained in the ER, newly synthesized APP preventing Aβ production. We are now generating several specific APP-directed antibody-derivatives (e.g. a recombinant full-length antibody, a FAB fragment and a single chain antibody). These tools will be utilized in several ways. Upon purification of the secreted recombinant proteins, the full-length antibody, the FAB fragment and the single chain antibody could be used for passive immunization. The genes for their expression could be used for virus-mediated gene therapy (*in vivo* gene therapy). Cells stably expressing these reagents could be used for ex-vivo gene therapy. This approach is being tested in a collaborative project with the group of Patrick Aebischer at the EPFL. Briefly, we prepared stable C2C12 cells producing high levels of the single chain antibody or of the FAB fragment. C2C12 have contact-inhibited growth. They can therefore be encapsulated in appropriate capillary tubes with protein-permeable walls. Capsules are then inserted in the brain of mice model of Alzheimer disease. Cells enclosed in the capsules will produce and secrete for several months in the brain of treated mice the antibody derivatives hopefully delaying disease onset.

• *Paganetti P. et al. / J Cell Biol 2005; 168:863-868.*

Details

Funding

Max Cloëtta Foundation, Medical Research Position.
2001-2006.

Foundation for Research on Neurodegenerative Diseases, Mimicking passive immunization in vitro to inhibit Aβ production.
2001-2008.

S. Salvatore Foundation: Research Grant for IRB Research.
2005-2008

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2006

Swiss National Center of Competence in Research on Neural Plasticity and Repair, Single chain antibodies and FAB fragments to modulate Aβ production.
2002-2007

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3100A0-107578/1 / *2006-2008.*

Synapsis and Bangerter-Rhyner Foundation, Intrabodies to regulate Aβ production.
2006-2008

Collaborations

Randy J. Kaufman / Medical School, University of Michigan, Ann Arbor, USA.

Paolo Paganetti / Novartis, Basel, Switzerland.

Lloyd Ruddock / Department of Biochemistry, University of Oulu, Finland.

Visiting Scientists

Masatoshi Hagiwara / Kyoto University, Kyoto, Japan.

Publications

2005

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Moremen K. W. and Molinari M. / *Curr Opin Struct Biol 2006; 16:592-599*

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Heberth D.N., Molinari M.
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Book Chapters

"The secretory capacity of a cell depends on the efficiency of endoplasmic reticulum associated degradation."
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Lectures and Seminars

2005

"Protein folding and quality control in the endoplasmic reticulum...and an approach to modulate production of the toxic amyloid-beta peptide."
University of Brescia, Brescia, Italy / *21.02.2005*

"b-site specific intrabodies to decrease and prevent generation of Alzheimer's Ab peptide."
NCCR Neural Plasticity and Repair Symposium, Kartause Ittingen, Switzerland / *4-5.03.2005*

Cell Biology Meets the Immune System: Molecular Aspects of Host Pathogen Interactions, International Titisee Conference"Protein quality control in the endoplasmic reticulum."
Titisee. Germany / *6-10.04.2005*

"EDEM and ERAD in protein folding and quality control." Protein Maturation and Function.
Oulu, Finland / 25-27.04.2005

"β-site specific intrabodies to decrease and prevent generation of Alzheimer's Aβ peptide."
Neurobiology meeting, Institute Mario Negri, Milano, Italy / *14.06.2005*

FEBS-IUBMB Meeting, Protein Folding and Transport in Health and Disease,"EDEM and ERAD in protein folding and quality control."
Bucharest, Romania / *28.06-2.07.2005*

Basic Virology Course. "The folding of viral glycoproteins in the endoplasmic reticulum."
Institut Pasteur, Paris, France / 6.09.2005

International Symposium on Life of Proteins, "Glycoprotein quality control without UGT1."
Awaji Island, Japan / *30.10-3.11.2005.*

2006

USGEB Meeting 2006, "Protein folding in the Endoplasmic Reticulum." Friedrich-Miescher Award 2006.
Geneva,Switzerland / *23-24.02.2006*

Neuroscience Seminar, "From protein folding and quality control to a novel approach to reduce production of the toxic amyloid-beta peptide."
EPFL Lausanne, Switzerland / *28.03.2006*

First Meeting of the Association Medicine Anti-Aging, "The Endoplasmic Reticulum: a factory for proteins and diseases."
Lugano, Switzerland / *6.04.2006*

Seventh International Calreticulin Workshop, "Functions and Dinamics of ER/SR Proteins". "Protein folding and quality control in chaperone deficient cell lines."
Niagara Falls, Ontario, Canada / *22-24.04.2006*

151st Meeting of the Società Ticinese Scienze Naturali, "The Endoplasmic Reticulum: a factory for proteins and diseases."
Bellinzona, Switzerland / *6.05.2006*

Merilän Kartano Meeting,"Protein Quality Control in UGT1-deleted cells."
Utajärvi, Finland *8-10.10.2006*

Science et Cité, Musica e Molecole. "Dall'Uomo alle Molecole alla Musica."
Bellinzona, Switzerland / *19.06.2006*

Basic Virology Course, Institut Pasteur, "The folding of viral glycoproteins in the endoplasmic reticulum."
Paris, France / *7.09.2006*

8th Jenner Glycobiology and Medicine Symposium, "N-Glycan processing determines the fate of folding-competent and -defective glycoproteins."
Scripps Research Institute & Neuroscience Research Institute California, La Jolla, USA
17-20.09.2006

Simposio Invecchiamento, Demenza e Ricerca. "Approaches to reduce production of the toxic peptide Aβ."
Lugano, Switzerland / *21.09.2006*

Simposio Alzheimer 1906-2006 Un secolo di scoperte, "Alzheimer 100 anni dopo: traguardi e prospettive della ricerca medica."
Locarno, Switzerland / *28.09.2006*

2007

"Protein folding and quality control in the endoplasmic reticulum."
Venetian Institute of Molecular Medicine,Padua, Italy / *15.01.2007*

"Recombinant antibodies and antibody fragments to human APP: Production in mammalian cells, use to mimic passive immunization protocols and secretion from encapsulated cells".
NCCR Neural Plasticity and Repair, Zurich, Switzerland / *14.02.2007*

"Immunotherapies to reduce the production of the toxic amyloid beta peptide" Research Award Aetas 2007.
Swiss Foundation for Ageing Research, Geneva, Switzerland / *21.03.2007*

"La malattia di Alzheimer."
Giornate Autogestite Liceo di Bellinzona, Bellinzona, Switzerland / *29.03.2007*

Signal Transduction Course, "Another example of Signal Transduction: from the ER into the cell cytosol."
Curzùtt, Switzerland / *4-5.06.2007*

FASEB Summer Research Conference "From Unfolded Proteins in the Endoplasmic Reticulum to Disease". "Baseline autophagy regulates constitutive glycoprotein disposal from the ER by determining rapid EDEM1 turnover."
Indian Wells, California, USA / *28.07-2.08.2007*

63rd Harden Conference-Protein folding and assembly in vitro and in vivo,"N-glycan processing in endoplasmic reticulum quality control."
St Martin's Coll., Ambleside, UK / *18-23.08.2007*

World Conference of Stress,"Protein folding quality control and degradation in the ER: the role of N-glycans."
Budapest, Hungary / *23-26.08.2007*

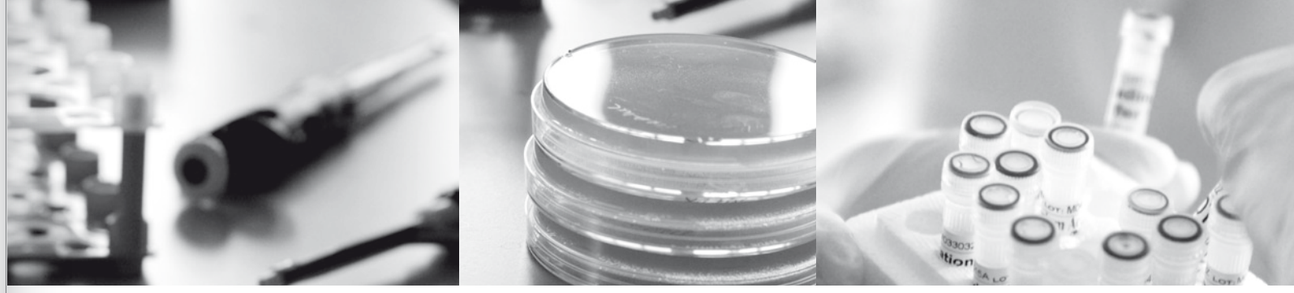
CELL MIGRATION

Marcus Thelen obtained his PhD in 1985 from the University of Bern studying the bioenergetics of mitochondria.

He was a PostDoc at the Theodor-Kocher Institute in Bern from 1985 to 1989, later from 1989 to 1991 at the Rockefeller University in New York. In 1992 he was awarded a START fellowship from the Swiss National Science Foundation and headed a laboratory at the Theodor-Kocher Institute, University of Bern.

Since 2000 he is a group leader at the IRB. In 1994 he obtained the Venia Docendi and later in 2001 was awarded an Honorary Professor title from the University of Bern where he still is member of the Medical Faculty.

Prof. Thelen has published more than 60 papers. His research covers several aspects of biochemistry, cell biology and human immunoogy. His focus is on signal transduction in chemokine receptor mediated cell activation and migration.



Signal Transduction

Cell migration is essential for development and survival of multicellular organisms. Generally, single cells move along cues to reach their destination. The process requires polarization, i.e. the formation of a morphological distinct front and a rear end, of the cell along the axis of attraction. The intracellular pathways which sense the attracting signal and transduce it into remodeling of the actin cytoskeleton and the sequential activation of adhesion molecules is a central focus of our research.

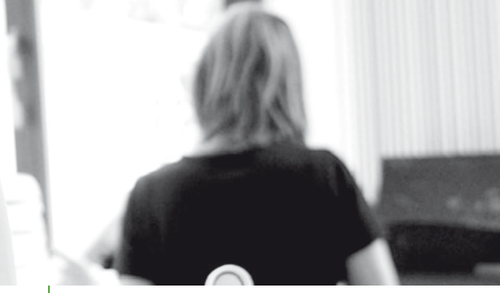
Leukocyte trafficking is largely regulated by chemokines and their respective receptor. Migration of cells expressing subsets of receptors is further controlled by spatially restricted secretion of chemokines. The mechanism is important for immune homeostasis, but is also essential for acute and chronic immune responses such as inflammation. In addition, some cancer cells appear to use the chemokine systems for metastasis.

Laboratory

Group Leader: Marcus Thelen, *PhD, 2000*

Members: Sylvia Thelen, *PhD 2000* • Tiziana Apuzzo, *PhD student, 2006* • Ulrike Naumann, *PhD student, 2006* • Silvia Volpe, *PhD student, 2006.*



CXCR4 associated proteins and their role in cell specific receptor function.

Tiziana Apuzzo, Elena Palmesino and Marcus Thelen.

As a prototype, we investigate coupling of the G-protein coupled chemokine receptor CXCR4 to downstream signal transduction pathways. The receptor, which is widely expressed in many tissues, plays a pivotal role in embryonic development, in immune regulation, and in tumor growth and metastasis. Several lines of evidence indicate that signaling downstream of CXCR4 depends on the cellular environment. Furthermore, cell specific signal transduction must be regula-ted in close proximity of the receptor. To this end we investigate the receptor proteome to elucidate coupling to downstream signal transduction pathways. We have developed a solublization protocol that maintains the heptahelical receptor protein in its native and active conformation. We then use mass spectrometry to identify receptor associated proteins in different cells and following stimulation. Finally functional assays are used to elucidate the functional relevance of the observed interactions.

• Palmesino, E., et al. / *Immunobiology 2006; 211:377-389*

RDC1, an orphan receptor with similarities to chemokine receptors.

Ulrike Naumann, Simona Infantino and Marcus Thelen.

Phylogenetic analyses and expression patterns suggested that the so called orphan receptor RDC1 may serve as a chemokine receptor. We have generated a specific monoclonal antibody and could demonstrate that the receptor binds the chemokine CXCL12 (SDF-1). The receptor was renamed to CXCR7. We further characterized the expression of CXCR7 on leukocytes and found that mature plasmacytoid dendritic cells can release a factor that is distinct from CXCL12. The putative ligand induces receptor internalization on peripheral blood CD19^{+} B cells. On these cells expression of CXCR7 correlates with the ability to differentiate into antibody producing cells.

The physiological role of CXCR7 remains to be established. We are currently investigating functional responses elicited by the receptor and are further characterizing ligand receptor interactions.

• Balabanian,K., et al. / *J Biol Chem. 2005; 280:35760-35766* • Infantino,S., et al. / *J Immunol 2006; 176:2197-2207*

Cell migration: analysis of receptor coupling to actin polymerization.

Silvia Volpe and Marcus Thelen.

Shallow gradients of chemoattractants with a concentration difference of less than 2% fully promote the migration of mammalian cells. We set up video time-laps microscopy to study receptor distribution and activity during chemotaxis in such gradients. Intramolecular fluorescence resonance energy transfer (FRET) of cyan fluorescent protein / yellow fluorescent protein (CFP/YFP) tags are used to monitor receptor activity. Similarly coupling of the chemotactic receptor to downstream signal transduction pathways will be monitored by FRET analysis. The experimental setup allows monitoring recruitment of proteins relative to the axis of polarization and possible direct interactions.

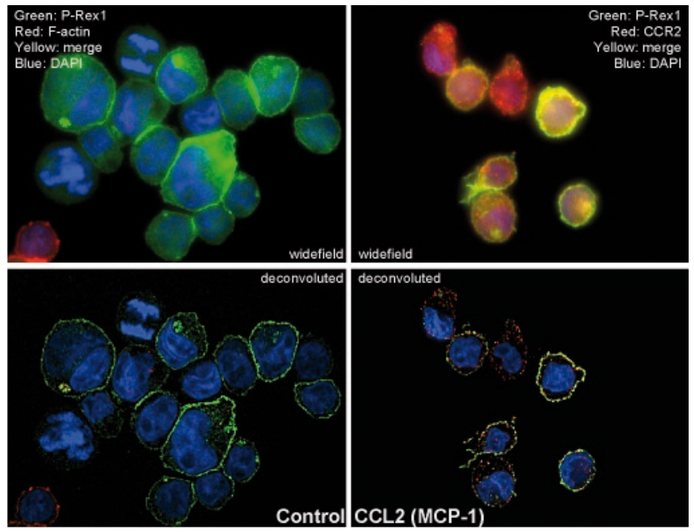
Role of the guanine nucleotide exchange factor P-Rex1 in chemokine receptor mediated signaling.

Sylvia Thelen and Marcus Thelen.

The small RhoGTPases Rac and CDC42 are assumed to play a key role in agonist-stimulated actin polymerization and are therefore considered as critical regulators of cell migration. Selective and spatially -restricted activation of the widely expressed RhoGTPases is essential for polarized actin polymerization during migration. This can be achieved through the receptor-mediated stimulation of specific guanine nucleotide exchange factors (GEF) which act upstream of small GTPases. We observed that following treatment of cells with chemokines the GEF P-Rex1 undergoes rapid translocation to the plasma membrane (Fig 1).

Translocation to the plasma membrane correlates with the appearance of transient posttranslational modifications, which can be detected on western blots. The electrophoretic mobility shifts are sensitive to phosphatase treatment, suggesting that P-Rex1 undergoes agonist-dependent phosphorylation. Current investigations aim to elucidate the pathways leading to P-Rex1 phosphorylations and to determine the functional consequences of the modifications.

• Welch,H.C., et al. / Curr. Biol 2005; 15:1867-1873. • Barber,M.A., et al. / J. Biol. Chem. Epub. August 13th 2007; doi:10.1074/jbc.M701877200.



THP-1 cells stably transfected with GFP-Tagged P-Rex1. Cells were treated with medium or 100 nM CCL2 for 90 sec, fixed and stained.

Funding

European Community- MAIN:Targeting Cell Migration in Chrinic Inflammmation
FP6-NoE LSHG-CT-2003-502935, BBW-Nr. 03.0441-1 / *2003-2008*

European Community- INNOCHEM:Innovative Chemokine-based Therapeutic Strategies for Autoimmunity and Chronic Inflammation
FP6-LSHP-CT-2005-518167 / *2005-2010*

Swiss National Science Foundation: Chemokine receptors and signal transduction pathways in leucocytes.
3100A0-112214 / *2006-2009*

Swiss National Science Foundation, "R'Equip" Grant for confocal microscope.
2007

San Salvatore Foundation: Analysis of Chemokine receptor CXCR4 interacting proteins in different tissues.
2006-2009

Novartis Stiftung für Medizinisch-Biologische Forschung, Chemokine mediated signal transductiuon.
2006

Collaborations

Ronen Alon / Department of Immunology, Wolfson Building, Weizmann Institute of Science, 76100 Rehovot, Israel.

Françoise Bachelerie and Dr. Fernando Arenzana-Seisdedos / Institut Pasteur Unité de Pathogénie Virale Moléculaire, F-75724 Paris, France.

Peter Gierschik and PD. Dr. Barbara Moepps Institute of Pharmacology and Toxicology, University of Ulm, Germany.

Hanno Langen / RCMG, Hoffamnn-La Roche Ltd, Basel, Switzerland.

Martin Lohse and Dr. Moritz Bühnemann Institute of Pharmacology and Toxicology, University of Würzburg, Germany.

Bernd Müller / Department of Biologie I, Ludwig-Maximilian University, Munich, Germany.

Paul O'Shea / School of Biology, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom.

Antal Rot / Novartis Institutes for BioMedical Research GmbH & Co KG, A-1235 Vienna, Austria.

Matgot Thome / Department of Biochemistry, University of Lausanne, Switzerland.

H.C. Welch / The Inositide Laboratory, Babraham Institute, Babraham Research Campus, Cambridge CB22 3AT, UK.

Publications

2005

The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes.
Balabanian K., Lagane B., Infantino S., Chow K. Y., Harriague J., Moepps B., renzana-Seisdedos F., Thelen M., and Bachelerie F. / *J Biol Chem 2005; 280:35760-35766*

P-rex1 regulates neutrophil function.
Welch H. C., Condliffe A. M., Milne L. J., Ferguson G. J., Hill K., Webb L. M., Okkenhaug K., Coadwell W. J., Andrews S. R., Thelen M., Jones G. E., Hawkins P. T., and Stephens L. R. / *Curr Biol 2005; 15:1867-1873*

2006

Expression and regulation of the orphan receptor RDC1 and its putative ligand in human dendritic and B cells.
Infantino S., Moepps B., and Thelen M.
J Immunol 2006; 176:2197-2207

Differences in CXCR4-mediated signaling in B cells.
Palmesino E., Moepps B., Gierschik P., and Thelen M. / *Immunobiology 2006; 211:377-389*

2007

Membrane translocation of P-Rex1 is mediated by G protein beta gamma subunits and phosphoinositide 3-kinase.
Barber M. A., Donald S., Thelen S., Anderson K. E., Thelen M., and Welch H. C. / *J Biol Chem 2007; Aug 13 Epub*

Bcl10 Controls TCR- and FcγR-Induced Actin Polymerization.
Rueda,D., O.Gaide, L.Ho, E.Lewkowicz, F.Niedergang, S.Hailfinger, F.Rebeaud, M.Guzzardi, B.Conne, M.Thelen, J.Delon, U.Ferch, T.W.Mak, J.Ruland, J.Schwaller, and M.Thome. 2007 / *J Immunol 178:4373-4384.*

Lectures and Seminars

2005

"Is CXCR7 (RDC1) a Chemokine Receptor."
RWTH Aachen, Germany / *07.09.2005*

"Is CXCR7 (RDC1) a Chemokine Receptor."
MDC Berlin, Germany / *13.10.2005*

9th International Dahlem Symposium of Cellular Signal Recognition and Transduction, "Regulatory Circuits in Chemokine Receptor-Mediated Signal Transduction."
Berlin, Germany / *14.10.2005*

"Innochem Kick Off Meeting, RDC1: an orphan receptor?"
Milano, Italy / *21.11.2005*

2006

"RDC1: an orpahn receptor?"
Polyphor Basel, Switzerland / *06.02.2006*

Chemotactic Cytokines, Gordon Research Ronference, Chairman presentation "Must CXCR7 follow the fate of Pluto?"
Centre Paul Langevin, Aussois, France
17-22.09.2006

"Analysis of Signaling Networks downstream of G-protein Coupled Receptors."
University of Ulm, Ulm, Germany / *27.10.2006*

"Chemokine Receptor Signal transduction: Common and distinct features."
University of Rome, Roma, Italy / *20. 11. 2006*

2007

"CCXR7/RDC1 versus CXCR4: Common and Distinct Features of Chemokine Receptors."
University of Bern, Bern, Switzerland
29.01.2007

"Common and distinct Signal Transduction by Chemokine Receptors."
University of Ferrara, Ferrara, Italy / *06.07.2007*

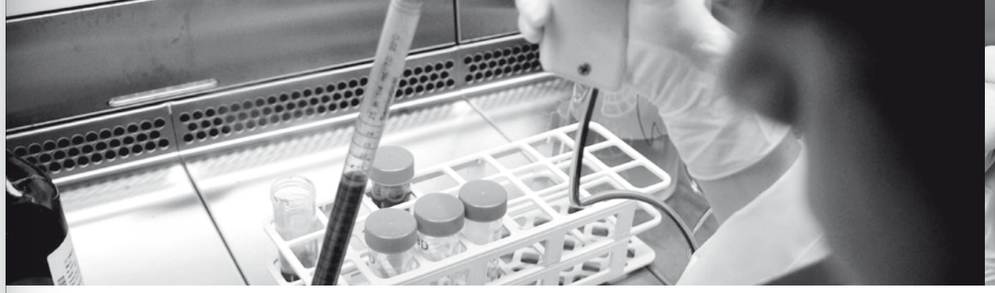
DEVELOPMENT

Fabio Grassi earned his degree in Medicine at the University of Pavia and a PhD. in Microbiology at the University of Milan. He worked at the University of Umeä in Sweden, the Institut Pasteur and Hopital Necker in Paris, the San Raffaele Scientific Institute in Milan.

He has been a Special Fellow of the Leukemia & Lymphoma Society at Harvard Medical School in Boston. He is Associate Professor of Biology at the University of Milan.

In September 2002, he joined the IRB as head of the T Cell Development lab.

His research is focused on molecular as well as cell biology of T cell differentiation in the thymus, signal transduction of the T cell in secondary lymphoid organs and in T cell mediated inflammatory conditions.



T Cell Development

The experiments performed in the lab are principally focused on the characterization of signal transduction pathways at different developmental stages of the murine T cell. A first aim is to define signaling microdomains, which are involved in transducing the signal by the pre-T cell receptor (pre-TCR) and promote T cell as well as thymus development. A second aim pursued in the lab is to characterize signaling pathways controlled by Ca^{2+} during immunopathological T cell responses leading to inflammation and tissue destruction. Another topic studied by our group is the impact of T cell activation during an inflammatory response on hematopoiesis and bone homeostasis.

Laboratory

Group Leader: Fabio Grassi, *MD, PhD, 2002.*

Members: Anna Casati, *PhD student, 2005* • Denise Ferrera, *PhD student, 2003* • Ursula Schenk, *PhD, 2005* • Micol Ferro, *Undergraduate student, 2005.*

Signaling microdomains in pre-T cell development.

Denise Ferrera and Fabio Grassi.

Developmental regulation of gene expression ensures commitment and progression of immature T cell precursors along the αβ or γδ differentiation pathways. Commitment to the αβ pathway is characterized by the transition of $CD4^-8^-$ double negative (DN) thymocyte to the $CD4^+8^+$ double positive (DP) stage. This transition requires recombination at the TCR loci and is promoted by expression of the pre-TCR in the plasma membrane. The pre-TCR is palmitoylated and is enriched in low-density plasma membrane fractions in which lipid rafts are preferentially recovered. Lipid rafts are enriched in proteins playing a crucial role in most upstream events in pre-TCR signaling, including the src-family kinase lck and the adapter LAT. We hypothesized that pre-TCR affinity for rafts could contribute to the ligand independency of pre-TCR signaling. However, palmitoylation of pTα juxtamembrane cysteine was shown to be dispensable for DP transition of thymocytes and specific charged residues in the extracellular portion of the pTα chain were shown to mediate receptor oligomerization independently of rafts localization, thereby questioning the relevance of rafts partitioning in pre-TCR signal initiation.

A peculiar feature of immature thymocyte is to express in the plasma membrane the endoplasmic reticulum (ER) resident chaperone calnexin (CNX) in association with CD3 chains. Plasma membrane CNX/CD3 complex partitions to glycolipid enriched microdomains (rafts) as the pre-TCR, albeit to a much lesser extent. Once cross-linked with anti-CD3 antibodies CNX/CD3 complexes prominently accumulate into rafts and drive transition of pre-TCR deficient thymocyte to the DP stage mimicking at both cellular and molecular level all events induced by pre-TCR expression. Analogously to the pre-TCR, surface CNX is palmitoylated. We designed different mutant CNX isoforms capable of shuttling CD3 to rafts with different efficiencies. Strong but not weak translocation of CD3 into rafts determined pre-TCR-like signaling and simulated β selection *in vivo*. Inclusion of cytoplasmic tail from pre-Tα (pTα) chain in place of CNX cytoplasmic tail increased pre-TCR signaling efficiency. These results strongly support the view that signaling by oligomerized pre-TCR initiates in lipid rafts and that β selection is implemented by pTα cytoplasmic tail. Furthermore, the use of mutant CNXs isoforms might be useful in dissecting the signaling pathways exploited by the pre-TCR to exert its pleiotropic develop mental function.

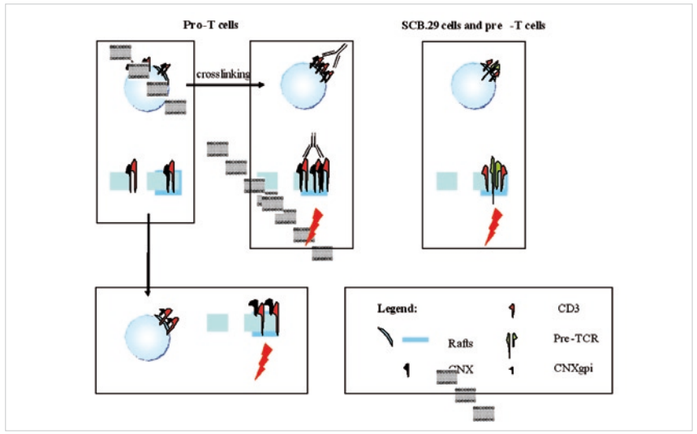


Figure 1: *Model of T cell development triggered by CD3 raft partition. In pro-T cells, mab-mediated crosslinking of CNX/CD3 complex causes an enhanced partition of the complex within rafts and promotes signaling. A raft-targeted CNX shuttles CD3 to rafts and initiates signaling. In pre-T cells, the pre-TCR/CD3 complex constitutively partitions within the raft fraction and transduces a signal.*

Regulation of signal transduction in the T cell.

Ursula Schenk, Micol Ferro and Fabio Grassi.

Calreticulin (CRT) is the main Ca^{2+} buffering protein in the endoplasmic reticulum (ER) and its deficiency in mice is embryonically lethal because of altered cardiac development. To study the impact of CRT deficiency on T cell function, we reconstituted recombinase-2-deficient (RAG-2)/common γ chain double knock-out (DKO) mice with fetal liver hemopoietic progenitors (FLP) from *crt*$^{-/-}$ as well as *crt*$^{+/+}$ embryos. RAG/γ chain DKO mice reconstituted with *crt*$^{-/-}$ FLP display alopecia and blepharitis starting at week 7 after reconstitution with 20% of mice progressing to a wasting disease. Cells displaying markers of activation and constitutively secreting cytokines in both the CD4 and CD8 αβ T cell lineages were increased in peripheral lymphoid organs. These cells could derive from inefficient deletion of autoreactive T cells in the thymus; however sensitivity to apoptosis of thymocytes in several assays was unaltered with respect to *crt*$^{+/+}$ cells. A functional defect of the regulatory T cell subset was ruled out by efficient inhibition of anti-CD3 mitogenic response by sorted *crt*$^{-/-}$ $CD4^+25^+$ cells.

Crt$^{-/-}$ cells display improved IL-2 secretion upon TCR cross-linking and responsiveness to suboptimal stimulation that does not promote survival of *crt*$^{+/+}$ T cells. This aberrant control of TCR signaling is likely involved in the immunopathology observed in *crt*$^{-/-}$ fetal liver chimeras. We investigated Ca^{2+} dynamics in *crt*$^{-/-}$ versus *crt*$^{+/+}$ T cells following TCR αβ stimulation and found that cytosolic Ca^{2+} elevations were significantly perturbed in *crt*$^{-/-}$ cells. *Crt*$^{-/-}$ cells displayed reduced Ca^{2+} release from intracellular stores and increased frequencies of Ca^{2+} elevations after TCR stimulation likely representing inefficient inactivation of Ca^{2+} release activated Ca^{2+} (CRAC) channels. Indeed, mitochondrial Ca^{2+} buffering was more efficient in *crt*$^{-/-}$ cells and had a direct effect on CRAC channels inactivation. This mechanism could likely contribute to prolonged responsiveness of *crt*$^{-/-}$ T cells and more generally be involved in aberrant T cell activation during inflammatory disease.

Impact of T cell activation on bone marrow homeostasis.

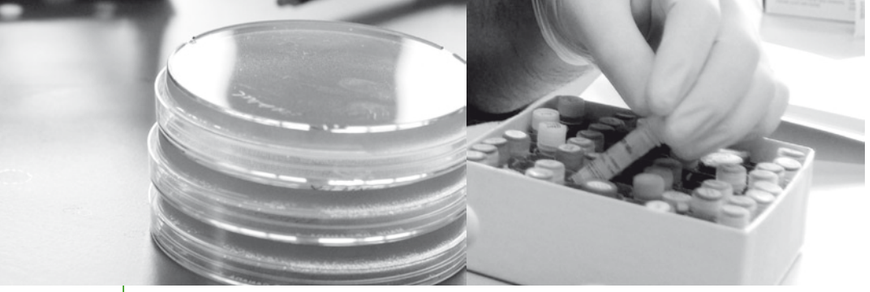
Anna Casati and Fabio Grassi.

Infection and inflammatory signals promotes granulopoiesis over lymphopo-iesis. The consequences of T cell activation on bone marrow homeostasis are poorly defined. We could show the selective depletion of immature but not mature bone marrow B cells and early skewing of hematopoietic stem cells to the granulocyte-monocyte differentiation pathway in two models of T cell dependent tissue inflammation, namely calreticulin-deficient fetal liver chimeras and inflammatory bowel disease.

The differential influence on B cell progenitors versus mature B cells supports the dependence on distinct microenvironment resources of the two B cell pools.

Peripheral effector/memory T cells positively correlated with granulocyte-monocyte progenitors independently of tissue inflammation. In contrast, peripheral T cell activation positively correlated whereas T cell activation associated with tissue injury negatively correlated with differentiation to the megacaryocyte/erythroid pathway. Consistently, the CCAAT/enhancer-binding protein (C/EBP) α and C/EBPβ transcription factors were upregulated upon immunopathological T cell activation. This study is aimed at defining selective effects of effector/memory T cells associated or not with tissue inflammation on bone marrow homeostasis.

Figure 2: *Immunological synapse between a Fura2 loaded DO11.10 transgenic T cell and an OVA peptide loaded dendritic cell.*



Details

Funding

European Community - MUGEN: Integrated Functional Genomics in Mutant Mouse Models as Tools to Investigate the Complexity of Human Immunological Disease.
FP6 –NoE-LSHG-CT-2005-005203 / *2005-2010.*

Fondazione Ticinese per la Ricerca sul Cancro, Role of molecular chaperones in pre-T cell receptor function and leukemogenesis.
2004-2007.

Roche Research Foundation: Role of calreticulin in the T cell receptor mediated signaling.
2006-2007.

Swiss National Science Foundation: Molecular requirements and subcellular routing of signals in pre-T cell development and leukemogenesis.
3100A0-104237/1 / *2004-2007.*

Collaborations

Michela Matteoli / Department of Pharmacology, University of Milan, Milan, Italy.

Marek Michalak / Department of Biochemistry, University of Alberta, Edmonton, AB, Canada.

Francesca Sanvito / Immunohistochemistry of Rodents Unit, San Raffaele Research Institute, Milan, Italy.

Eugenio Scanziani / Department of Pathology, Faculty of Veterinary Medicine, University of Milan, Milan, Italy.

Camillo Ricordi / Diabetes Research Institute, University of Miami, Miami, FL, USA.

Elisabetta Traggiai / Center of Excellence for Biomedical Research, University of Genoa, Italy.

Anna Villa / Human Genome Department, Institute of Biomedical Technologies, CNR, Milan, Italy.

Mariapia Abbracchio / Department of Pharmacological Sciences, University of Milan, Italy.

Publications

2005

Involvement of Prep1 in the alphabeta T-cell receptor T-lymphocytic potential of hematopoietic precursors.
Penkov D., Di Rosa P., Fernandez Diaz L., Basso V., Ferretti E., Grassi F., Mondino A., and Blasi F.
Mol Cell Biol 2005; 25:10768-10781

2006

Regulation of peripheral T cell activation by calreticulin.
Porcellini S., Traggiai E., Schenk U., Ferrera D., Matteoli M., Lanzavecchia A., Michalak M., and Grassi F. / *J Exp Med 2006; 203:461-471*

Notch1-dependent lymphomagenesis is assisted by but does not essentially require pre-TCR signaling.
Campese A. F., Garbe A. I., Zhang F., Grassi F., Screpanti I., and von Boehmer H. / *Blood 2006; 108:305-310*

2007

A hypomorphic R229Q Rag2 mouse mutant recapitulates human Omenn syndrome.
Marrella V., Poliani P. L., Casati A., Rucci F., Frascoli L., Gougeon M. L., Lemercier B., Bosticardo M., Ravanini M., Battaglia M., Roncarolo M. G., Cavazzana-Calvo M., Facchetti F., Notarangelo L. D., Vezzoni P., Grassi F., and Villa A.
J Clin Invest 2007; 117:1260-1269

Lectures and Seminars

2005

Venetian Institute of Molecular Medicine, "Impact of calreticulin deficiency on lymphocyte function".
Padua, Italy / *13.10.2005*

2006

"Calcium dependent shaping of T cell activation."
Institute A. Fleming, Vari, Greece / *04.04.2006*

"αβ T cell development."
Institute Pasteur, Paris, France / *06.11.2006*

"Signal transduction in T cell development."
San Raffaele Scientific Institute, Milan, Italy
22.11.2006

"Regulation of T cell responsiveness and inflammation by calcium signaling."
University of Pavia, Italy / *12.12.06*

STEM CELLS

Markus G. Manz received his degree in Medicine 1995 from the Eberhard-Karls-University in Tuebingen, Germany.

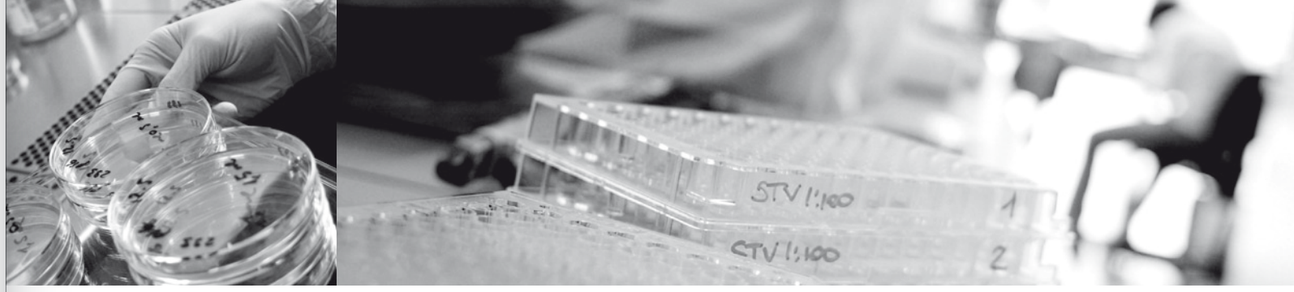
Between 1995 and 1999, he trained in internal medicine at the Tuebingen Medical School, from 1999 to 2001 he worked as a postdoctoral fellow in the laboratory of Irving Weissman at Stanford, USA, and in 2002 he became group leader at IRB.

From 2004 to 2006 he finished his hematogy/oncology training at the University of Tuebingen, while maintaining the IRB lab as an Associate Group Leader.

Since September 2006 he is group leader at the IRB and associated hematologist at the Oncology Institute of Southern Switzerland (IOSI), Bellinzona.

Markus Manz scientific interest focuses on hematopoiesis and immune system development. His research is driven by the urge to understand basic mechanisms of immune system maintenance and regeneration in steady-state and disease, a knowledge that eventually will be valuable for the development of new strategies to interfere with this process, e.g in states of infection, immunodeficiency, autoimmunity, hemato-lymphoid cancers, or in solid organ and hematopoietic cell transplantation.

In 2004 he received the prestigious Artur-Pappenheim Award of the German Society of Hematology and Oncology.



Hematopoiesis

Throughout life, a small fraction of hematopoietic stem cells (HSCs) self-renew in the bone marrow and generate all cells of the hemato-lymphoid system, a system with very high cellular turn-over. Because of its ready accessibility, hematopoiesis is currently one of the best studied mammal adult stem cell differentiation systems, and is likely paradigmatic for other physiologic (e.g. liver, skin, central nervous system) and pathologic (tumors, leukemia) stem cell regenerated compartments. Beyond its model character for basic research, hematopoietic stem cell transplantation (formerly bone marrow transplantation) is so far the only successfully working clinical stem cell therapy, mostly applied for the treatment of malignant hematologic disease or immunodeficiencies. Also, hematopoietic stem cells currently provide the major gateway for clinical gene therapy.

The hierarchically structured, unidirectional differentiation process from HSCs to terminally differentiated cells involves progressive loss of self-renewal ability, proliferation capacity, and lineage differentiation potentials. In my laboratory, we are studying regulation of physiologic and pathologic hemato-lymphopoieis in steady-state, and inflammatory conditions, as well as in neoplasia in both mice and men.

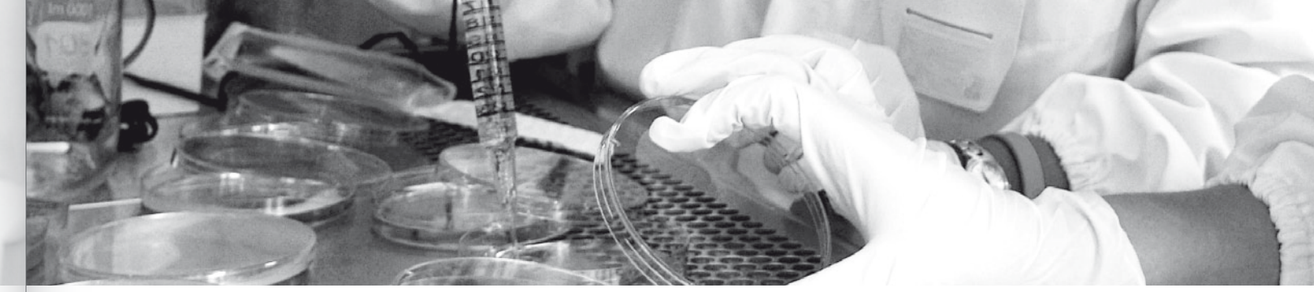
Our hypothesis is that HSC maintenance, subsequent commitment and expansion to different cellular lineages is largely controlled externally, depending on varying demand. To evaluate this, we characterize and isolate human and murine hematopoietic stem- and progenitor cells as well as bone marrow stroma cell components (mesenchymal stroma cells, MSCs), study and subsequently modify their transcriptional profile, and test their responsiveness to physiologic stimuli and to pharmacologic compounds in both *in vitro* and *in vivo* assays.

In depth understanding of physiologic maintenance and differentiation pathways from HSCs to mature cells of the hematopoietic system will eventually provide new insights and improved therapeutic methods to treat hematopoietic and immune system diseases.

Laboratory

Group Leader: Markus G. Manz, *MD, 2002.*

Members: Patrick Ziegler, *PhD, 2006* • Michael A. Schmid, *PhD student, 2006* • Steffen Boettcher, *MD student, 2006* • Aya Onai, *PhD, 2004-2006* • Nobuyuki Onai, PhD, *2004-2006* • Roxane Tussiwand, *PhD student, 2003-2006* • Sekhar Boddupalli, *PhD student,2007* • Dior Kingston, *PhD student, 2007* • Hitoshi Takizawa, *PhD, 2007.*



Ongoing Projects

Development and function of natural type I interferon-producing cells and dendritic cells.

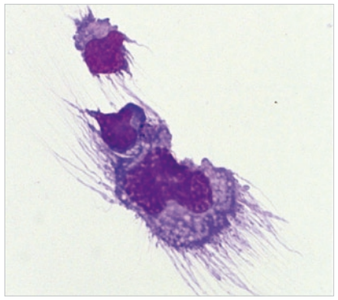


Figure 1:Cytospin of in vitro generated mouse dendritic cells

Involvement of natural interferon producing cells and dendritic cells in juvenile idiopathic arthritis.

Marco Gattorno, Laurie Chicha, and David Jarrossay.

Recent data suggest that two prototype autoimmune diseases, systemic lupus erythematosus and rheumatoid arthritis are mainly driven by distinct cytokines, interferon (IFN)-α and tumour necrosis factor (TNF)-γα, respectively. We thus investigated the presence and characteristics of natural type I IFN-α producing cells (IPCs), as well as IFN-α γand TNF-α expression in peripheral blo-od and at sites of inflammation in juvenile idiopathic arthritis (JIA) patients.

Interestingly, we found that IPCs were enriched in SF MNCs compared with PB MNCs in all JIA patients. Influenza-induced, but no spontaneous IFN-α release was detected from SF IPCs, and serum and SF IFN-α levels were not elevated. Nonetheless, in synovial tissue IFN-α producing cells accumulated at inflammatory lymph-follicular-like structures, while TNF-α producing cells were mostly found at the lining and sublining layers. Thus, these data suggest that besides TNF-αγ-expressing cells, IFN-αγ-producing IPCs are involved in initiation, maintenance or regulation of the inflammatory response in JIA.

Ongoing studies aim to evaluate in more depth systemic and involved joint pathology in correlation to onset, continuation, and disease flares in these young patients.

Regulation of natural type I interferon-producing and dendritic cell development and homeostasis.

Michael A. Schmid, Nobuyuki Onai, Aya Onai, Roxane Tussiwand, Sekhar Boddupalli and Dior Kingston.

Flt3-ligand is a non-redundant cytokine in type-I interferon-producing cell (IPC) and dendritic cell (DC) development (as shown in Flt3-ligand deficient mice), and IPC and DC differentiation potential is confined to $Flt3^+$-hematopoietic progenitor cells. We tested over-expression of human Flt3 in $Flt3^-$- and $Flt3^+$-progenitors and found that flt3 expression and stimulation rescues and enhances their IPC and DC differentiation potential, respectively. In $Flt3^-$ megakarycyte/erythrocyte restricted progenitors (MEPs), enforced Flt3-signaling induced transcription of IPC-, DC-, and GM-development affiliated genes and activates differentiation capacities to these lineages. Moreover, ectopic expression of Flt3 downstream transcription factors STAT3 or PU.1 in $Flt3^-$ MEPs instructs differentiation into IPCs, DCs, and myelomonocytic cells. This suggests an environmental Flt3-ligand cytokine driven model, where Flt3 acts as the earliest inducer

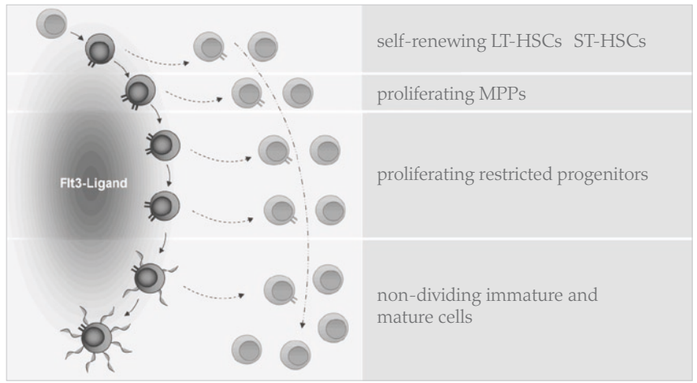


Figure 2: Proposed "Flt3-Licence" working model for steady-state natural IPC and DC development from early hematopoietic progenitor cells. Bold arrows represent continuous strong Flt3-ligand stimulation leading to IPC and DC development, dashed arrows represent more frequent competing signals, leading to alternative lineage outcomes (from Onai et al., Ann N Y Acad Sci. 2007 Mar 14; [Epub ahead of print]).

and a constant enhancer of steady-state IPC and DC generation.

Our working hypothesis is thus that DC development, as e.g. erythropoiesis or thyroid hormone production, is regulated via positive and/or negative feedback mechanisms at the hematopoiesis-immune system interface to ensure appropriate DC regeneration upon demand in steady-state and inflammation.

We address regulation of DC differentiation by a) identifying sequential critical early dendritic cell commitment steps in mouse and human hemato-lymphopoiesis (isolation and full characterization of candidate clonal common dendritic cell committed progenitors, CDPs); b) studying dendritic cell development relevant cytokine/cytokine receptor expression and function in steady-state and immunological challenges *in vivo* in mice (generation and evaluation of Flt3-ligand and GM-CSF double deficient mice, and $Flt3\text{-ligand}^{-/-}GM\text{-}CSF^{-/-}$ mice that in addition lack M-CSFR in their hematopoietic compartment, generation and evaluation of Flt3 and Flt3-ligand reporter mice); c) testing the influence of inflammatory immune stimuli on dendritic cell development *in vitro* and *in vivo* (mapping of conserved pathogen receptor expression along dendritic cell developmental pathways, and testing of DC development under the influence of pathogen associated factors *in vitro* and *in vivo*); and d) interfering pharmacologically with dendritic cell development and evaluating resulting impact on *in vivo* immune responses (vaccination, experimental autoimmune encephalitis, and graft versus host disease).
Understanding mechanisms of DC regeneration will not only provide knowledge about how these rare but essential regulators of the immune system are produced in steady-state and inflammation, but likely also will generate some fundamental insight into how conserved signals of invading pathogens might be integrated in early hematopoiesis in order to mount efficient immune responses. Furthermore, knowledge about physiological regeneration of DCs will be valuable for the development of new strategies to interfere with this process in order to either enhance or ameliorate DC guided immune responses, e.g in states of immunodeficiency, autoimmunity, or in solid organ and hematopoietic cell transplantation.

Human-Hemato-Lymphoid System Rag2-/-gc-/- mice.

Steffen Boettcher, Roxane Tussiwand, Hitoshi Takizawa, Patrick Ziegler and Dior Kingston.

Because ethical restrictions limit *in vivo* studies of the human hematolymphoid system, substitute human to small animal xenotransplantation models have been employed. Existing models, however, sustained only limited development and maintenance of human lymphoid cells and rarely produce immune responses.

We found that intrahepatic injection of $CD34^+$ human cord blood cells into conditioned newborn $Rag2^{-/-}\gamma c^{-/-}$ mice leads to de novo development of B, T, dendritic, and natural type I interferon-producing cells; formation of structured primary and secondary lymphoid organs; and production of some functional immune responses.

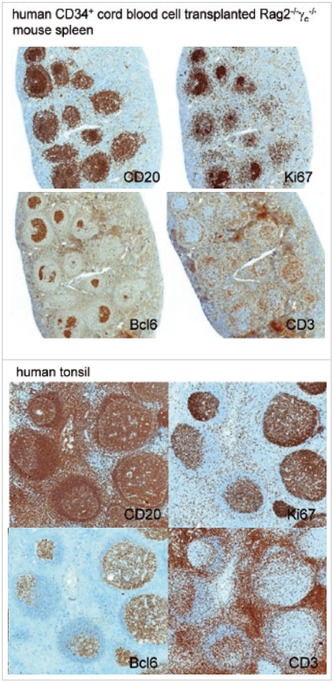
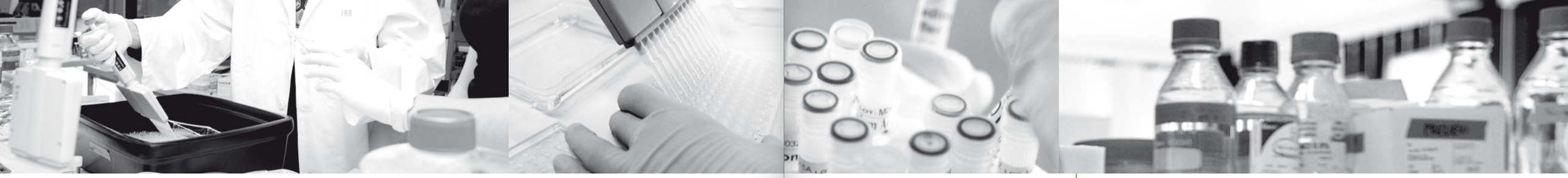


Figure 3: Lymphoid-Tissue Similarities in Human-Hemato-Lymphoid-System Mice and Men. The upper panel shows spleen sections of a newborn intrahepatic CD34+-cord-blood-cell-transplanted Rag2-/-Il2rg-/- mouse. The lower panel shows control stains on human tonsils for comparison. (from M.G. Manz, Immunity. 2007 May 25;26 (5):537-541

Using this model, we are currently studying a) human T and B cell differentiation and selection, b) human *in vivo* IPC, DC, and Langerhans cell differentiation and maintenance, c) human hematopoiet-ic stem and progenitor cell maintenance and differentiation, d) infection, immune responses, and therapeutic interventions in human specific virus



infections (EBV and HIV) *in vivo*, and e) engraftment, differentiation and therapy of human malignant hematopoietic cells.

Together with the group of Roberto F. Speck from the University of Zürich, we tested these mice as a new model for HIV infection. We infected human hemato-lymphoid system mice intraperitoneally with either CCR5 tropic or CXCR4 tropic HIV-1 strains. Irrespective of co-receptor selectivity, HIV RNA plasma copies peaked at 2-4 weeks after infection, comparable to HIV infection in man. Thereafter, viremia mostly stabilized at lower levels and was maintained for up to 190 days, the longest time followed. As in man, developing human $CD4^+$ thymocytes in mice are mostly CXCR4-positive, but lack CCR5 expression, while peripheral $CD4^+$ T-cells express CXCR4 and/or CCR5. Thymic HIV infection was detected upon CXCR4 tropic infection, while secondary lymphoid organ infection occurred in both CXCR4- and CCR5-tropic virus infected animals. In both CXCR4- and CCR5-tropic strain infected animals productively HIV-infected cells, i.e. HIV-$p24^+$ cells, were mostly $CD3^+$ and only occasionally non T cells such as $CD68^+$ macrophages. In some mice with high numbers of productively infected cells, syncytium formation occurred in both spleen and lymph nodes, a process observed in brains and lymphoid tissue of HIV infected individuals, likely associated with high viral replication, spreading, and CD4 cell loss. Overall, CXCR4-tropic HIV infection lead to more rapid blood $CD4^+$ cell loss than CCR5-tropic infection, reminiscent of CXCR4-tropic emergence of HIV strains in late-stage human HIV disease. One out of 25 infected mice mounted a HIV-specific IgG response detectable by standard clinical assays. Furthermore, although based on limited data, we did not detect robust HIV-specific T cell responses, as determined by IFN-γ detection upon *in vitro* re-stimulation, in line with other concomitant reports. Thus, although specific immune responses are observed, they thus far lack robustness, i.e. are not predictable in frequency and levels, prohibiting e.g. at this stage efficient pre-clinical vaccine testing. However, as is, this model will be valuable to study virus-induced pathology and to evaluate new, non-adaptive immunity dependant approaches aiming to fight HIV.

As long-term human HSC maintenance, human myeloid differentiation, and adaptive immune cell developmental selection and function is limited in $Rag2^{-/-}\gamma c^{-/-}$ mice, we are working in a collaborative, Gates Foundation Grant Challenges supported project (including the laboratory of R. Flavell, Yale University, and S. Stevens, Regeneron) to replace some mouse cytokines and MHC components by human counterparts.

In addition, we try to support human hemato-lymphopoiesis by co-transfer of human mesenchymal stroma cells that might be involved in building an appropriate environment/niche for human hemato-lymphopoietic cells.

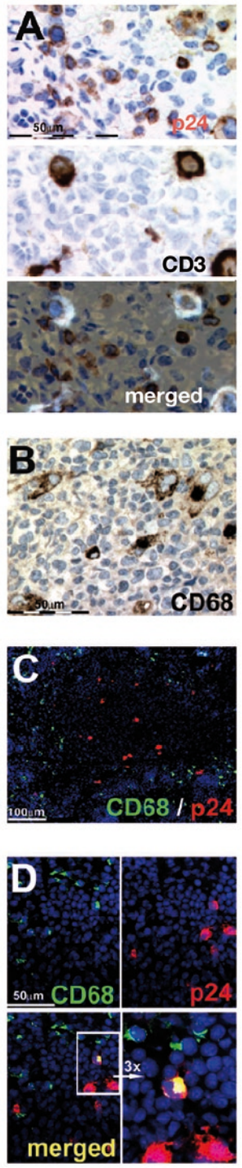


Figure 4: HIV p24+ cells are mostly human CD3+ cells and only occasionally non-T cells such as CD68+ macrophages. (A) *Histologies show consecutive spleen sections stained with antibodies against human CD3* (Upper) *and p24* (Middle) *and a merged presentation of both* (Lower) *in a YU-2-infected animal 18 days after infection.* (B)*Anti-human CD68 staining on paraffin-embedded material.* (C) *Merged anti-CD68 (green), HIV-p24 (red), and DAPI (blue) staining of cryoembedded spleen (6-µm sections) showing that p24+ cells mainly localize in white pulp areas (darker area; see also Fig. 4*B), *whereas CD68+ cells mostly localize at adjacent margins and red pulp areas.* (D) *Consecutive spleen cryosection staining and respective merged presentation showing a rare CD68 and p24 double-positive cell (yellow). The far-right image shows a 3x enlargement of the area with the double-positive cell.* (B–D) *Representative spleen sections from a YU-2-infected animal 23 days after infection (from Baenziger et al., Proc Natl Acad Sci U S A. 2006 Oct 24;103(43):15951-6).*

Details

Funding

Swiss National Science Foundation: Development and characterization of a mouse model to study the human hematopoietic and immune system in vivo.
3100-10222 / *2003-2005.*

Swiss National Science Foundation: In vivo evaluation of HIV infection using mice that carry a human adaptive immune system.
3100A0-108352/1 / *2005-2007*

European Community: Targeted Research Project - STROKEMAP: Multipotent adult progenitor cells to treat stroke.
LSHB-CT-2006-037186 / *2006-2009*

European Community - DC-THERA: Dendritic Cells and Novel Immunotherapies; Network of Excellence.
FP6 –NoE-LSHG-CT-2005-005203 / *2005-2010 2007-2009,*

Swiss National Science Foundation: Regualtion of dendritic cell development from hematopoietic stem and progenitor cells.
310000-116637/1 / *2007-2010*

Oncosuisse: An in vivo study on the stem cell origin of chronic myeloproliferative disease.
OCS-02019-02-2007 / *2007-2009*

Bill and Melinda Gates Foundation Grand Challenges in Global Health Program: A humanized mouse model to evaluate live attenuated vacchine candidates.
GC#4 / *2005-2010.*

Collaborations

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R. Flavell / Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT, USA.

M. Gattorno / Second Division of Pediatrics, 'G. Gaslini' Institute for Children and University of Genoa, Genoa, Italy.

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T. Heidenreich / Eberhard-Karls Universität Tübingen, Germany.

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L. Leoncini / Pathology, University of Siena, Italy.

L. Mazzucchelli / Pathology, Locarno, Switzerland.

M. Merad / Mount Sinai School of Medicine, N.Y, USA.

J.-C. Piffaretti / Istituto Cantonale di Microbiologia Bellinzona, Switzerland.

R.C. Skoda / University of Basel, Switzerland.

T. Sparwasser / Department of Microbiology, Technical University of Munich, Germany.

R. Speck / Department of Infectious Disease, University Hospital Zürich, Switzerland.

R. Stanley / Albert Einstein College of Medicine, NY, USA.

S. Stevens / Regeneron, Terrytown, N.Y., USA.

M. Suter / Bavarian-Nordic, Munich, Germany.

C. Verfaillie / Stem Cell Institute, University of Leuven, Belgium.

R. Weissert / Department of Neurology, University of Tübingen Medical School, Germany, and Merck-Serono, Geneva, Switzerland.

Publications

2005

Human Adaptive Immune System Rag2-/-{gamma}c-/- Mice
Chicha L., Tussiwand R., Traggiai E., Mazzucchelli L., Bronz L., Piffaretti J. C., Lanzavecchia A., and Manz M. G. / *Ann NY Acad Sci 2005; 1044:236-243*

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Macchiarini F., Manz M. G., Palucka A. K., and Shultz L. D. / *J J Exp Med 2005; 202:1307-1311*

2006

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Baenziger S,Tussiwand R, Schlaepfer E, Mazzucchelli L, Heikenwalder M, Kurrer MO, Behnke S, Frey J, Oxenius A, Joller H, Aguzzi A, Manz MG*, and Speck RF*, / *Proc Natl Acad Sci U S A. 2006 Oct 24;103(43):15951-6. Epub 2006 Oct 12. *equal contribution.*

2007

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Gattorno M, Chicha L, Gregorio A, Ferito F, Rossi F, Jarrossay D, Lanzavecchia A, Martini A, Manz MG / *Rheumatology (Oxford) 2007 Apr; 46(4):657-65. Epub 2006 Nov 3.*

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Onai N, Obata-Onai A, Schmid MA, Manz MG *Ann N Y Acad Sci. 2007 Mar 14; [Epub ahead of print].*

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Book Chapters

Manz MG. Chapter 1: Dendritic Cell and Natural Type I Interferon-Producing Cell Development, pp 1-7, Antigen Presenting Cells and the Eye (2007)
Eds. M. Zierhut, H.G. Rammensee, and J.W. Streilein Informa Healthcare USA, Inc., New York, NY

Baenziger et al.: Human T cell development and HIV infection in Human Hemato-Lymphoid System Mice, in "International Workshop on Humanized Mice."
In Press"Current Topics in Microbiology and Immunology" (Springer)

Lectures and Seminars

2005

"Immunoreconstitution-of mice and men."
Biochemie, Universität Tübingen, Germany
10.01.2005

3rd EAACI-Davos Meeting, "Dendritic cell development."
Davos, Switzerland / *05.02.2005*

Cellular Therapies 2005, "Regulation of dendritic cell development from hematopoietic progenitor cells."
Regensburg, Germany / *18.03.2005*

"Immunoreconstitution-of mice and men."
Anatomisches Institut Universität Bern, Schweiz / Baxter BioScience, Wien, Austria
19.01.2005

"Human adaptive immune system mice."
Mikrobiologie, Universität Erlangen / *26.04.2005*

The Nikolas Symposium on Langerhans Cell Histiocytosis, "Dendritic cell development."
Athens, Greece / *07.05.2005*

1st EMBL Monterotondo (6th EMBL) International PhD Symposium, "Immunoreconstitution-of mice and men"
Rome, Italy / *14.05.2005*

Vortragstitel: "Immunoreconstitution-of mice and men."
University of Texas Southwestern, Dallas, USA
31.05.2005

Vortragstitel: "Dendritic cell development-new options for immunomodulation?"
Chochin Meeting on Dendritic Cells, Paris, France / *24.09.2005*

"Immunoreconstitution-of mice and men"
GSF, Munich, Germany / *13.10.2005*

"Dendritic cell development."
Department of pediatric Immunology, Istituto Gaslini, Genoa, Italy / *27.10.2005*

Second Conference on Focus in Paediatric Haematology-Oncology, "Dendritic cell deve lopment."
Sestri Levante, Italy / *29.10.2005*

"A mouse model to evaluate live attenuated vaccine candidates."
Bill and Melinda Gates Foundation, Seattle, USA / *21.11.2005*

2006

"huAIS-RG mice-new options to test human lymphotropic viruses?"
ASM, Washington DC, USA / *26.02.2006*

"huAIS-RG mice-opportunities and limitations."
Baylor Institute for Immunology Research, Dallas, TX, USA / *09.05.2006*

"huAIS-RG mice-opportunities and limitations."
Mount Sinai School of Medicine, New York, USA / *10.05.2006*

"huAIS-RG mice-new options to test human lymphotropic viruses?"
American Association of Immunologist, Washington DC, USA / *13.05.2006*

"huAIS-RG mice-new options to test human lymphotropic viruses?"
Childrens Hopital & Harvard Medical School, Boston, USA / *15.05.2006*

"Dendritic Cell Homeostasis."
Institut für Mikrobiologie, TU Munich, Germany
29.06.2006

"Human Immune System Mice."
1st International MUGEN Conference, Athens, Greece / *11.09.2006*

"Flt3 in dendritic cell development."
"Haematopoietic Stem Cells VI" Universität Tübingen, Germany / *16.09.2006*

First International Workshop on Humanized Mice, "HIV infection in humanized mice."
Tokyo, Japan / *12.10.2006*

"HIV infection in humanized mice."
Riken Institute,Yokohama, Japan / *13.10.2006*

"Humanized mice."
Medizinische Hochschule Hannover, Germany
29.11.2006

2007

"Human Adaptive Immune System Mice-New Options to Study Human Lymphotropic Infections in vivo."
Keystone Symposium „Immunological Intervention in Human Disease", Big Sky Montana, USA / *10.01.2007*

"Humanized Mice-Opportunities to study Human Hematopoiesis."
Heidelberger Akademie der Wissenschaften, Germany / *02.02.2007*

"Tumor-Stammzellen: Neue Perspektiven in der Tumorbiologie."
Universität Tübingen, Habilitations-Vortrag, Germany / *06.02.2007*

"Human lymphotropic infections in mice."
Heinrich-Pette Institut Hamburg, Germany
02.03.2007

"DC Homeostasis."
DC-Crest St. Moritz, Switzerland / *28.03.2007*

SGAI-SSAI-Meeting, "DC Homeostasis."
Basel, Switzerland / *19.04.2007*

"Humanized mice in vaccine development."
Wellcome Trust, Cambridge, UK / *04.05.2007*

"Humanized mice."
EFI Meeting, Barcelona, Spain / *07.05.2007*

"Dendritic cell development and homeostasis."
Rolduc Meeting on T cell Biology, The Netherlands / *21.05.2007*

"Human Hemato-Lymphoid System Mice."
Annual retreat of the Infection Biology Program at Karolinska Institutet Stockholm, Sweden
30.08.2007

HIV-AIDS

Jeremy Luban earned an M.D. at the College of Physicians and Surgeons, Columbia University in New York, in 1987. He underwent clinical training in Internal Medicine and Subspecialty fellowship training in Infectious Diseases, as well as postdoctoral training in Biochemistry in the laboratory of Stephen Goff.

He became a Professor at Columbia University with dual appointments in the Department of Medicine (1993) and the Department of Microbiology (1995). In addition to teaching medical students, physicians, and PhD graduate students he served as director of the Columbia-Rockefeller Center for AIDS Research.

His laboratory focuses on host factors that regulate HIV-1 replication and confer innate immunity to this deadly virus.

Among the HIV-1 regulatory factors discovered by his lab are the human proteins cyclophilin A and TRIM5.

Dr. Luban became a group leader at the Institute for Research in Biomedicine and relinguished his position at Columbia University in 2007.

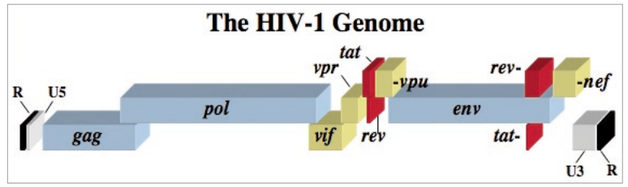
Viral replication, pathogenesis, and immunity.

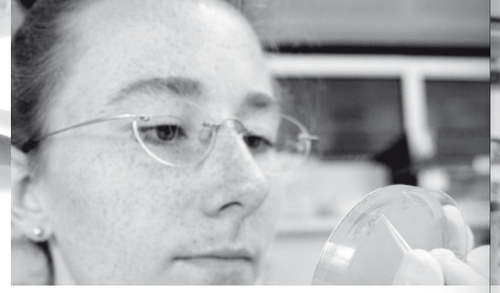
Study of retroviruses has led to major advances in fundamental biology, most notably the discovery of oncogenes and the modification of the central dogma. In this spirit, we investigate mechanisms of HIV-1 replication and pathogenesis with the goal of advancing understanding of the basic workings of the cell. Through the development of genetic and biochemical screens we attempt to identify cellular factors of importance to HIV-1 and, more generally, to cell physiology. In effect, we exploit HIV-1, using the virus to elucidate mechanisms of cell cycle progression and cytokinesis, signal transduction and cytokine expression, protein folding and degradation, as well as pathogen recognition and antigen presentation. Our research is basic in nature but by shedding light on mechanisms of HIV-1 replication and immune system evasion we hope to contribute to the development of drugs and vaccines that target this virus.

Laboratory

Group Leader: Jeremy Luban, *MD, 2006.*

Members: Christina Helbig, *PhD Student, 2006* • Susanne Märtens, PhD, *2007* • Martha Neagu, *PhD, MD, student, 2006* • Silvia Olivari, *PhD, 2007* • Thomas Pertel, *PhD student, 2006* • Nadia Rahm, *PhD student, 2006* • Caterina Strambio de Castillia, *PhD, 2005.*



Research Projects

Host factors that regulate HIV-1 infectivity.

Christina Helbig, Martha Neagu, Silvia Olivari, Thomas Pertel, Nadia Rahm.

Our lab discovered that the peptidyl-prolyl isomerase cyclophilin A (CypA) embraces an exposed, proline-rich loop on HIV-1 capsid (CA) and acts after virion membrane fusion with human cells to increase HIV-1 infectivity. HIV-1 CA is similarly greeted by CypA soon after entry into non-human primate cells, where, paradoxically, the interaction decreases HIV-1 infectivity. Our attempts to understand the effects of CypA on HIV-1 infectivity led to the discovery of TRIM5, a gene that confers innate resistance to retroviral infection in primates, including humans. TRIM5 can be thought of as a cytoplasmic receptor which recognizes CA-specific determinants on the retroviral core encasing the viral genomic RNA. It establishes a paradigm of innate immunity in which an invading multiprotein complex, rather than double-stranded RNA or lipopolysaccharide, is recognized by a specific receptor. Ongoing experiments will attempt to understand the mechanism by which TRIM5 restricts retroviruses, how CypA modulates HIV-1 sensitivity to TRIM5 via effects on CA structure, and how TRIM5 interacts with other immune system components to control HIV-1 in people.

Automated, Quantitative, Real-Time Tracking of HIV-1 Within Human Cells.

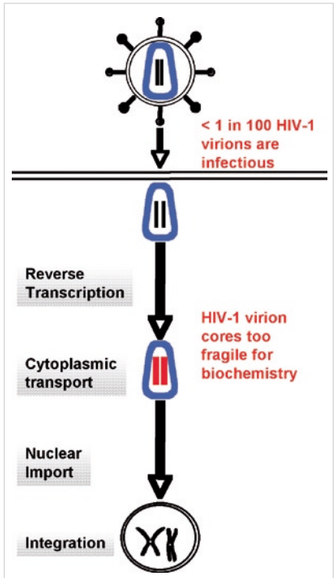
Caterina Strambio de Castillia, Nadia Rahm, and Jeremy Luban.

After the HIV-1 virion membrane fu-ses with a target cell, the virion core is delivered into the cytosol where a DNA copy (cDNA) of the viral RNA genome is produced by reverse transcription (RT).

The trigger for the initiation of RT is unknown, as is the sub-cellular location where RT occurs. Similarly, the mechanisms by which these sub-virion complexes are transported within the cytoplasm are not known.

Viral cDNA then translocates through the nuclear pore where it is ligated to host cell chromosomal DNA by the viral integrase protein (IN) to establish the provirus.

How viral cDNA is translocated through the nuclear pore, and how integration sites are selected in the chromatin, is not known. Attempts to establish functional correlates for experimental observations concerning these early steps in the HIV-1 life cycle have been hindered by the fragile nature of HIV-1 replication intermediates in cells and the low HIV-1 infectivity-to-particle ratio (< 1:100). In collaboration with Ivo Sbalzarini (ETHZ) and Mario Valle (CSCS) we are developing methods for directly visualizing HIV-1 subvirions in real-time as they traverse the cell. We will track the rare particles that successfully establish a provirus. Properties that distinguish these replication-competent particles will be identified. Such information will be invaluable for the rational.



design of anti-HIV-1 drugs targeting these essential steps of the retrovirus life cycle.

Small animal model for HIV-1 replication and pathogenesis.

Martha Neagu, Patrick Ziegler, Daniel Venetz, Thomas Pertel, Mariagrazia Uguccioni, Markus Manz, and Jeremy Luban.

HIV-1 causes a fatal immune suppression (AIDS) in perhaps all who are infected with the virus. Ongoing efforts to develop new drugs that inhibit HIV-1 replication or retard AIDS progression would benefit from experimental systems that better reflect *in vivo* physiology. HIV-1 will not replicate in the cells of non-human primates other than chimpanzees. HIV-1 also will not replicate in rodent cells. Here in Bellinzona, Markus Manz demonstrated that immunodeficient mice can be reconstituted longterm with functional B, T, and dendritic cells derived from human umbilical cord blood. In collaboration wi-th Roberto Speck (Zurich) he showed that these animals are infectable with HIV-1. We have now established a facility in Bellinzona where these animals can be infected with HIV-1. Several questions are currently being addressed. Exactly which cells are infected in these animals? Can we transduce the hematopoietic stem cells that are used to reconstitute the mice using vectors that express antiviral factors? Will HIV-1 replication be blocked in animals that were reconstituted with cells transduced by these vectors? What is the role of non-essential HIV-1 accessory genes in the replication and pathogenisis of HIV-1 in this model system?

Details

Funding

National Institutes of Health: Gag and Cyclophilin in HIV Replication .
5R01AI036199-12, / *1994-2009*

National Institutes of Health: Cyclophilin A Function in the Immune System.
1R01AI059159-01A2, NIAID, NIH / *2006-2010*

Swiss National Science Foundation: TRIM5 and Innate Resistance to HIV-1.
3100AO-113558 / *2006-2009*

Boerhinger-Ingelheim Student Fellowship to Christina Helbig: Mechanism of HIV restriction by TRIM5.
2007-2009

Collaborations

Stefan Höglund / Department of Biochemistry, University of Uppsala, Sweden.

Kenneth Cornetta / Department of Medical and Molecular Genetics, Indiana University School of Medicine, USA.

Mark Muesing / Aaron Diamond AIDS Research Center, The Rockefeller University, New York, USA.

Klaus Strebel / Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, Maryland, USA.

Guido Kroemer / INSERM, U848, Institute Gustave Roussy, Villejuif, France.

Klas Blomgren / Institute of Neuroscience and Physiology, Göteborg University, Sweden.

Ineke Braakman / Department of Cellular Protein Chemistry, Univ. of Utrecht, Netherlands.

Markus Grütter / Department of Biochemistry, Univ. of Zurich, Switzerland.

Anna Cereseto / Scuola Normale Superiore, Pisa, Italy.

Ivo Sbalzarini / Institute of Computational Science, ETH Zurich, Switzerland.

Olivier Schwartz / Department of Virology, Institut Pasteur, Paris.

Mario Valle / Swiss National Supercomputing, Center, Manno, Switzerland.

Thumbi Ndung'u / Nelson R Mandela School of Medicine, University of KwaZulu Natal, Durban, South Africa.

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Jean-Luc Darlix / Ecole Normale Superieure, Lyon, France.

Frank Kirchhoff / Department of Virology, University of Ulm, Germany.

Ali Saib / Hôpital Saint-Louis, CNRS UMR 7151 - Universite Paris Diderot, France.*v*

Publications

2006

Cyclophilin, TRIM5, and innate immunity to HIV-1.
Sokolskaja E. and Luban J. / *Curr Opin Microbiol 2006; 9:404-408*

2007

Cyclophilin A, TRIM5, and resistance to human immunodeficiency virus type 1 infection.
Luban J. / *J Virol 2007; 81:1054-1061*

The Retroviral Restriction Factor TRIM5alpha.
Sebastian S. and Luban J. / *Curr Infect Dis Rep 2007; 9:167-173*

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Berthoux L., Sebastian S., Muesing M. A., and Luban J. / *Virology 2007; 364:227-236*

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Takeuchi H., Buckler-White A., Goila-Gaur R., Miyagi E., Khan M. A., Opi S., Kao S., Sokolskaja E., Pertel T., Luban J., and Strebel K.
J Virol 2007; 81:8080-8090

Cyclophilin A participates in the nuclear translocation of apoptosis-inducing factor in neurons after cerebral hypoxia-ischemia.
Zhu C., Wang X., Deinum J., Huang Z., Gao J., Modjtahedi N., Neagu M. R., Nilsson M., Eriksson P. S., Hagberg H., Luban J., Kroemer G.,and Blomgren K. / *J Exp Med 2007; 204:1741-1748*

Lectures and Seminars

2006

"Structural studies on TRIM5alpha"
Department of Biochemistry, University of Zurich, Zurich, Switzerland / *2006*

HIV-1 Pathogenesis Meeting, "Characterization of human TRIM5γmutants for CA-binding ability and retroviral restriction activity".
Keystone, Colorado, USA / *2006*

HIV-1 Pathogenesis Meeting, "CypA and TRIM5γindependently regulate HIV-1 infectivity."
Keystone, Colorado, USA / *2006*

2nd Swiss Workshop on Basic HIV Research, "Host factors that restrict HIV-1"
Morat/Murten, Switzerland / *2006*

4th International Conference on Innate Immunity, "Innate immunity to HIV-1."
Corfu, Greece / *2006*

"Cyclophilin, TRIM5 and innate resistance to HIV-1."
Department of Microbiology and Molecular Medicine, University of Geneva, Geneva, Switzerland / *2006*

FASEB Summer Res. Conf. on Virus Assembly, "HIV-1 CA and Cyclophilin A."
Saxtons River, VT, USA / *2006*

7th Annual Symposium on Antiviral Drug Resistance, "Cyclophilin and TRIM5 in Innate Immunity HIV-1."
Chantilly, VA, USA / *2006*

Nouvelles Pandemies, Les Comprendre, Les Combattre. Les "Dix-Neuvièmes Entretiens" du Centre Jacques Cartier, "Cyclophilin and innate immunity to HIV-1."
Lyon, France / *2006*

"Cyclophilin, TRIM5, and HIV-1."
Department of Virology, Universitätsklinikums Heidelberg, Germany / *2006*

Scientific Board Meeting, Swiss HIV Cohort Study,"Autoimmunity and AIDS pathogenesis."
Bern, Switzerland / *2006*

Second EuroStemCell International Conference: Advances in Stem Cell Research. "AIDS pathogenesis model in mice after adoptive transfer of hematopoietic elements derived from human embryonic stem cells"
Lausanne, Switzerland / *2006*

2007

"Effects of cyclosporine on HIV-1 Env incorporation into virions."
Cellular Protein Chemistry, Utrecht University, Netherland / *2007*

"TRIM5 and HIV-1 restriction."
San Raffaele Scientific Institute, DIBIT, Milan, Italy / *2007*

"Visualizing HIV-1 subvirion particles in living cells."
Scuola Normale Superiore, Pisa, Italy / *2007*

17th Challenge in Virology, "Innate immunity to HIV-1."
Saanen/Gstaad, Switzerland. / *2007*

Keystone Symposia. Molecular Pathogenesis of HIV-1. Keystone, "Screen for host factors required for TRIM5-mediated restriction."
CO. USA / *2007*

Retroviruses Meeting, "Screen for host factors required for TRIM5-mediated restriction."
Cold Spring Harbor, N.Y. USA. / *2007*

Retroviruses Meeting, "TRIM5alpha and the Type I interferon response."
Cold Spring Harbor, N.Y. USA. / *2007*

Retroviruses Meeting, "HIV-1 inhibition by engineered human TRIM-Cyp fusion proteins."
Cold Spring Harbor, N.Y. USA / *2007*

Retroviruses Meeting, "Real-time tracking of HIV-1 subvirion particles in acutely infected cells."
Cold Spring Harbor, N.Y. USA / *2007*

Targeting Replication and Integration of HIV-1, "Innate immunity to retroviruses"
Leuven, Belgium / *2007*

Host-Pathogen Interaction: The Role of Innate Immunity, "Cellular mechanisms for resistance to HIV-1 infection."
Bern, Switzerland / *2007*

Host-Pathogen Interaction: The Role of Innate Immunity, "Understanding the mechanism of HIV-1 restriction by TRIM5alpha"
Bern, Switzerland / 2007

"Cyclosporine analogues as HIV-1 inhibitors."
Debiopharm, Lausanne, Switzerland / *2007*

The Sidney E. Grossberg Lecturer, American Society for Virology, 26th Annual Meeting, "Cyclophilin, TRIM5, and Innate Immunity to HIV-1."
Corvallis, Oregon, USA / *2007*

"Innate immunity to viral pathogens."
Plum Island Animal Disease Center, US Department of Agriculture, Greenport, USA / *2007*

Third European Congress of Virology. "Host factors that restrict HIV-1 replication."
Nürnberg, Germany / *2007*

INFLAMMATION

Mariagrazia Uguccioni received a degree in Medicine from the University of Bologna (Italy) where she specialized in Haematology in 1994.

From 1993 to 2000 she was a member of the Theodor Kocher Institute, University of Bern (Switzerland), and since 2000 she is Head of the Chemokine Expression and Function Laboratory at the IRB.

She is Assistant Professor of Immunology at the School of Rheumatology, University of Bologna, since 2000.

Dr. Uguccioni's research has covered aspects of human haematology and immunology: chemokine activities, leukocyte activation and traffic, natural chemokine antagonists, and chemokine expression in human pathology.

Recently, her group is focusing on chemokine activities in human pathology and has identified a novel regulatory mechanism of leukocyte trafficking induced by synergy-inducing chemokines.

Chemokines: Tissue Expression, Function and Activity Modulation.

Our research interest remains focused on CHEMOKINE activities in physiology and pathology, with emphasis on mechanisms governing fine tuning modulation of their expression and activity. The breakdown in the control of leukocyte mobilization contributes to the pathogenesis of chronic inflammation as well as tumour development. Chemokines, are produced constitutively or upon specific induction in virtually all tissues of the human body (Figure 1). We have recently shown *(Paoletti et al., Blood 2005; Sebastiani et al., EJI 2005)* that non-ligand chemokines can enhance the activity of CCL19 and CCL21 on CCR7, and of CCL22 on CCR4, respectively. Western blot and binding experiments have shown the formation of heteromeric complexes suggesting these complexes as the cause of the observed synergism. Interestingly, the available structure and structure-function data, albeit scarce to date, collectively implicate residues in the first β-strand as mediators of heteromeric association and synergism (*Sebastiani et al., EJI 2005*). It is thus tempting to speculate that heteromeric chemokine complexes may mimic those homomeric dimers that form via association of their β-sheets, featuring an interface composed of the first β-strands. However, the molecular reasons as to why a heteromeric complex should be more active than a homomeric one remain, at present, completely obscure, and the analysis of this phenomenon is part of our ongoing research. Surface representations with electrostatic potentials of chemokines show similarities among selective agonists and known natural antagonists, thus indicating this analysis as an additional instrument for disclosing the potential of different chemokines as natural antagonist or synergy inducing molecules.

We have hypnotized that the synergism induced by heteromeric chemokine interactions may be a widespread phenomenon, positively regulating diverse chemokine activities such as chemotaxis, cellular adherence, receptor internalization, and protein kinase phosphorylation. Therefore, we are conducting additional *in vitro* studies to dissect in detail the mechanisms governing these activities.

Laboratory

Group Leader: Mariagrazia Uguccioni, *MD, 2000.*

Members: Maria Gabriela Danelon, *Technician, 2001* • Katrin Kuscher, *PhD student, 2004* • Tamara Visekruna, *PhD, 2005-2007* • Daniel Venetz, *PhD student, 2006* • Milena Schirarldi, *PhD student, 2007* • Denise Bottinelli, *undergraduated student, 2006.*




Chemokines in Pathology

There are clear indications for a role of chemokines in tumour biology, but the study of this area is still in its beginning. High-levels of multiple chemokines are expressed in tumour cells, tumour tissues and transformed cell lines. It has been suggested, and in some circumstances shown, that chemokines can act as growth factors and may have angio-genic or angiostatic properties. Tumours could also express chemokines in order to weaken immune defence. More recently it has also been shown that chemokines may mediate tumour cell migration and metastasis, suggesting chemokine receptors expressed on tumour cells as novel targets in anti-tumour treatment.

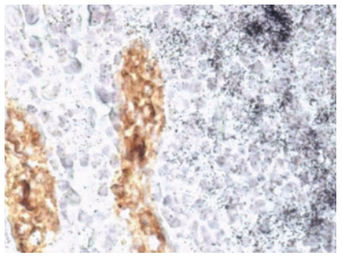
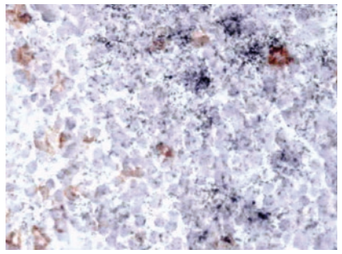



Figure 1: *Chemokine expression and cellular markers detected by double in situ/immunohistochemistry in a human tonsil. A Expression of CXCL9 (mRNA, black) and PNAd (borwn); B Expression of CXCL9 (mRNA, black) and CD20 (brown). Magnification 400x.*

Therapeutic potential of chemokine blockade

Research in the chemokine field has dramatically changed our understanding of leukocyte traffic in immune defence and disease, offering attractive perspectives for new therapeutic approaches in the treatment of chronic inflammation, infectious diseases, and tumours. The therapeutic potential of modulating chemokine activities was recognized from the beginning. Early studies, by our group and others, with receptor antagonists obtained by modifying the structure of natural chemokines proved the full validity of this concept, and low-molecular weight chemical compounds were recognised as prototypes for anti-chemokine drugs. Therefore, detailed studies on the expression and activity modulation of chemokines are crucial for assisting the developing of favourable anti-chemokine therapies in chronic inflammatory disease and tumours.

Impact of multiple chemokine expression in human disease.

Katrin Kuscher and Mariagrazia Uguccioni.

In situ experiments show the presence of a variety of chemokines in inflamed tissue. Our working hypothesis is that the concomitant expression of multiple chemokines may lead to enhanced leukocyte migration and functions. Our group has recently shown that several, non-ligand chemokines can synergise with CCR7 or CCR4 agonists. In contrast, few chemokines, such as CCL2, do not show any synergistic activity on these receptors. Noteworthy, another CCR2 agonist, CCL7, can synergize with either CCR7 or CCR4 agonists. In order to clarify if the phenomenon is receptor-dependent or chemokine-dependent we are studying the potential of non-ligand chemokines to synergize with CCR2-agonists on human monocytes expressing CCR2 but lacking CCR7. The concomitant exposure to CCL7 and non-ligand chemokines strongly enhances chemotaxis, while this synergistic effect is not observed when CCL2 is used.

In contrast, ERK phosphorylation induced by both CCR2 agonists can be powerfully enhanced by non-ligand chemokines.

CCR2 internalization and enzyme release are enhanced in the presence of the synergy-inducing chemokines. Co-immunoprecipitation experiments using CCR2 agonists and CCL19 or CCL21 did not show any heterocomplex formation, as it was previously reported.

As many chemokines are known to interact with glycosaminoglycans (GAGs), we used diffe-rent enzymes to shed GAGs from the cell surface. This treatment did not affect chemotaxis induced by CCL7 alone, nor the synergistic increase in the presence of CCL19 or CCL21. We are performing additional experiments for determining the mechanism of action of these synergy-inducing chemokines. The data, however, demonstrate that immediate functions of monocytes can be altered in the presence of non-ligand chemokines, while the effect of long term exposure of monocytes to multiple chemokines is under investigation.

• Paoletti S. et al. / *Blood 2005* • Sebastiani S. et al. / *EJI 2005*

Vaccines against human and simian immunodeficiency viruses: characterization of chemokine and chemokine receptor expression in lymphoid organs.

Maria Gabriela Danelon, Tamara Visekruna, Daniel Venetz, Denise Bottinelli and Mariagrazia Uguccioni.

We are pursuing our studies on the long-term effects of vaccines against the immunodeficiency viruses in the frame of two EU funded projects. In particular, the distribution of leukocytes according to their chemokinereceptor expression upon vaccination and/or after infection is analysed in secondary lymphoid organs by immunohistochemistry and *in situ* hybridisation. We show a strong expression of IFN-γ and CXCL9 induced by wild type SIV comparing with the expression of CXCL10 and CXCL11. The majority of these IFN-γ producing cells localize in the T area of the lymph node, and co-express CD56. Rhesus macaques lymph nodes vaccinated with attenuated SIV showed a significant decrease in IFN-γ production two weeks after challenge with wild type SIV, whereas no differences between animals challenged, or vaccinated and challenged were observed immediately after infection. Lymph nodes from HIV+ patients, undergoing highly active anti-retroviral therapy (HAART) which enables control of HIV replication and reduces HIV viraemia, revealed the same chemokine expression and CD56+ cell distribution as lymph nodes obtained from organ donors or from vaccinated and challenged animals. Vaccine-induced antiviral immune responses can control immunodeficiency virus replication within the lymph nodes which strongly correlate with down-modulation of IFN-γ and CXCR3 agonists (Visekruna et al., Manuscript in preparation). We have recently started a collaboration with two groups at the IRB (Markus Manz and Jeremy Luban) for the study of chemokine expression changes in tissues of HIV infected Rag2-/-γc-/-mice reconstituted with human CD34+ cord blood cells (huAIS-RG mice).

• Stahl-Hennig,C. et al. / *Front Biosci.,12:2107-2123, 2007* • Georgsson,G. et al. / *Neuropathol. Appl.Neurobiol., 2007.*

Chemokine expression in primary central nervous system lymphoma.

Maria Gabriela Danelon, Daniel Venetz and Mariagrazia Uguccioni.

Large B cell lymphomas are a heterogeneous group accounting for about 40% of adult non-Hodgkin lymphomas. Mature B-cell neoplasms are clonal proliferations of B cells at various stages of differentiation, ranging from naïve B cells to mature plasma cells. In contrast to T cell lymphomas, the anatomic site of B cell lymphoma has not been one of the major features defining these tumours. Recently, the location of large B cell lymphomas has been considered more carefully as the clinical outcome can depend on the sites of involvement. This feature is likely due to the fact that different organs are able to produce different chemoattractants as well as express different endothelial-associated proteins able to recruit selective cell subpopulations.

Our knowledge of the molecules guiding malignant B cells to extranodal sites, as well as on the function of chemokines in the tumour environment is at a very early stage. Scanty information is available so far on molecules involved in migration and on chemokine receptor expression in B cell lymphoma developing at extranodal sites. Our group has been the first to demonstrate inducible expression of CXCL13, a B cell chemoattractant, in relation to gastric lymphomas of the mucosa-associated lymphoid tissue (MALT), arising in association with *Helicobacter pylori*-induced gastritis and mucosal lymphoid aggregates, and in relation to non-Hodgkin B cell lymphoma arising within the CNS. At present only scattered information is available, apart for CXCL13, on chemokine expression and activity in B cell lymphomas at both nodal and extra-nodal sites.

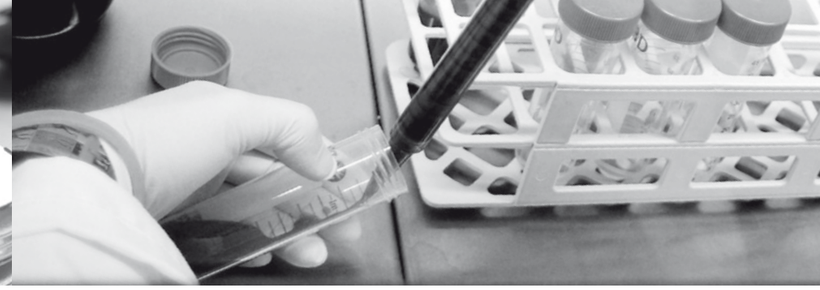
Nevertheless, there are clear indications for a role of chemokines in tumour biology. Finally, the recent demonstration of the presence of low amounts of B lymphocytes in normal brain opens new insights in the pathogenesis of PCNSL, and support the *in situ* study on the expression of homeostatic as well as of inflammatory chemokines.

HMGB1 and Chemokine Receptors in Chronic Inflammation.

Milena Schiraldi and Mariagrazia Uguccioni.

High-mobility group box 1 (HMGB1) is a protein loosely bound to the DNA that serves many purposes in the nucleus, facilitating transcription factor binding, nucleosome remodelling, and DNA repair process. HMGB1 contains two HMG-box domains (BoxA and BoxB) and an acidic C-terminus tail binding DNA and nucleosome without sequence specificity. It is one of the most abundant





proteins in the nucleus and it is crucial for life. Indeed, mice deficient of HMGB1 can fully develop, but die 24 hours after birth. HMGB1 can be passively released by necrotic, but not apoptotic, cells. Following apoptosis HMGB1 has been shown to be selectivity retained in the nucleus by condensed chromatin. It can also be secreted into the extracellular space by activated macrophages and mature dendritic cells (DCs) through an active process that requires its nuclear acetylation. Recently, it has been demonstrated that HMGB1 interacts also with chromatin fragments rich in CpG content, which are released by apoptotic cells. HMGB1-CpG complex containing oligodeoxynucleosides (ODNs) activates plasocytoid DCs and augments IFN-γ production through a mechanism dependent on the adaptor protein MyD88-TLR9 and RAGE (receptor for advanced glycation end product). This finding and the fact that HMGB1 is found in high amounts in sera of patients with autoimmune diseases, raises the possibility that HMGB1 may also contribute to the pathogenesis of autoimmune disorders characterized by the production of type I interferons.

While RAGE is considered the major receptor for HMGB1, two additional HMGB1 receptors have been identified so far: the toll like receptor (TLR) 2 and TLR4. In fact, transient transfection of TLR2 or TLR4 into human embryonic kidney 293 cells showed that HMGB1 induces cellular activation and NF-kB dependent transcription through TLR2 and TLR4. Moreover, co-precipitation of HMGB1 and TLR2 or TLR4 has been demonstrated. An additional association of intracellular HMGB1 with TLR9 has been shown, but this interaction remains to be elucidated. In collaboration with Marco E. Bianchi at the San Raffaele Scientific Institute (Milan, Italy), we are studying the migratory capacity of human monocytes and of cells transfected with different human receptors stimulated with HMGB1, in order to clarify the receptors and the signalling pathways involved in this process.

Details

Funding

European Community - MAIN:Targeting Cell Migration in Chrinic Inflammmation
FP6-E LSHG-CT-2003-502935, BBW-03.0441-1
2003-2008

European Community– TIPVAC:Explaining and Improving Efficacy of Targeted Immunodeficiency Virus-like Particle Vaccines against AIDS
FP6-LSHP-CT-2004-012116 / *2005-2007*

European Community – DEC-VAC: Development of a Dendritic Cell-targeted Vaccine against AIDS.
FP6-LSHP-CT-2005-018685 / *2005-2009*

European Community - INNOCHEM: Innovative Chemokine-based Therapeutic Strategies for Autoimmunity and Chronic Inflammation.
FP6-LSHP-CT-2005-518167 / *2005-20010*

OncoSuisse: Primary central nervous system lymphoma.
KSP OCS 01443-12-2003 / *2004-2006*

Swiss National Science Foundation: Impact of multiple chemokine expression in human disease.
3100A0-104237/1 / *2004-2007*

San Salvatore Foundation: Chemokine expression in extranodal lymphomas.
2005-2008

Max Cloëtta Foundation Student Fellowship to Daniel Venetz.
2006-2009

Collaborations

Francesco Bertoni / IOSI, Bellinzona, Switzerland.

Manfred P. Dierich / Institute for Hygiene, University of Innsbruck, Austria.

Andrea Facchini / University of Bologna, Italy.
Martin Lipp, Max-Delbrueck-Center for Molecular Medicine, Belin-Buch, Germany.

Costantino Pitzalis / GKT School of Medicine, University of London, United Kingdom.

Marc Parmentier / Université Libre de Bruxelles, Belgium.

Paul Raczs / Bernhard Nocht Institute for Tropical Medicine University of Hamburg, Germany.

Justine Smith and James Rosenbaum / Casey Eye Institute, University of Oregon, Portland, USA.
Ralph Steinman / Cellular Physiology & Immunology, Rockefeller University, New York, USA.

Ralf Igantius / Institute for Microbiology, University of Berlin, Germany.

Klaus Überla / Department for Molecular and Medical Virology, University of Bochum, Germany.

Maurilio Ponzoni and Claudio Doglioni / San Raffaele Institute, Milan, Italy.

Visiting Scientists

Paolo Dolzani / University of Bologna, Italy.

Antonio Manzo / William Harvey Research Institute, University of London, London, UK.

Eleonora Olivotto / University of Bologna, Italy.

Publications

2005

Systematic microanatomical analysis of CXCL13 and CCL21 in situ production and progressive lymphoid organization in rheumatoid synovitis.
Manzo A., Paoletti S., Carulli M., Blades M. C., Barone F., Yanni G., Fitzgerald O., Bresnihan B., Caporali R., Montecucco C., Uguccioni M., and Pitzalis C. / *Eur J Immunol 2005; 35:1347-1359*

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Sebastiani S., Danelon G., Gerber B., and Uguccioni M. / *Eur J Immunol 2005; 35:746-756*

A rich chemokine environment strongly enhances leukocyte migration and activities.
Paoletti S., Petkovic V., Sebastiani S., Danelon M. G., Uguccioni M., and Gerber B. O.
Blood 2005; 105:3405-3412

CD molecules 2005: human cell differentiation molecules.
Zola H., Swart B., Nicholson I., Aasted B., Bensussan A., Boumsell L., Buckley C., Clark G., Drbal K., Engel P., Hart D., Horejsi V., Isacke C., Macardle P., Malavasi F., Mason D., Olive D., Saalmueller A., Schlossman S. F., Schwartz-Albiez R., Simmons P., Tedder T. F., Uguccioni M., and Warren H. / *Blood 2005; 106:3123-3126*

2007

The central nervous system in mucosal vaccination of rhesus macaques with simian immunodeficiency virus Deltanef.
Georgsson G., Stahl-Hennig C., Tenner-Racz K., Uberla K., Stoiber H., Uguccioni M., Dierich M., Ignatius R., Steinman R. M., and Racz P.
Neuropathol Appl Neurobiol 2007

A single vaccination with attenuated SIVmac 239 via the tonsillar route confers partial protection against challenge with SIVmac 251 at a distant mucosal site, the rectum.
Stahl-Hennig C., Eisenblatter M., Franz M., Stoiber H., Tenner-Racz K., Suh Y. S., Jasny E., Falkensammer B., Ugucchioni M., Georgsson G., Baroni C., Dierich M. P., Lifson J. D., Steinman R. M., Uberla K., Racz P., and Ignatius R.
Front Biosci 2007; 12:2107-2123

Book Chapters

"Recruitment and localization of immune cells."
B. Moser and M. Uguccioni / *Enciclopedia Reference of immunotoxicology, Edited by Hans-Werner Vohr, Springer 2007; 315-321*

"Natural chemokine antagonism and synergism."
M. Uguccioni and B. Gerber / *In Chemokine Biology – Basic Research and Clinical Application. Volume I: Immunology of Chemokines. Edited by Bernhard Moser, Gordon L. Letts, Kuldeep Neote, Birkhäuser 123-134, 2006.*

"Lymphocyte homing and immunology of extranodal lymphoid tissues."
M.Uguccioni, J.J.Campbell, M.E. Kadin.
Text Book on Extranodal Lymphomas. Edited by Franco Cavalli, Harald Stein. 2007 (in press).

Lectures and Seminars

2005

"Chemokines direct cell migration".
Istituto di Ematologia e Oncologia Medica "L. e A. Seràgnoli."
Bologna / *12-16.06.2005*

Seminar: "The chemokine network".
Universita' di Tor Vergata, Roma / *11.2005*

2006

XXV EAACI Congress, "Tuning Chemokine Activities."
Vienna / *10-14.06.2006*

"Chemokine expression in autoimmunity."
Istituto di Reumatologia, Bologna / *06.2006*

2007

SIICA Meeting, "Natural chemokines regulate leukocyte trafficking."
Trieste / *6-9.06.2007*

"Chemokine guide leukocyte traffic"
Istituto di Reumatologia, Bologna / *06.2007*

AIBT Course, "Chemokines in Bone Marrow Transplantation."
Roma / *2007*

IMMUNITY

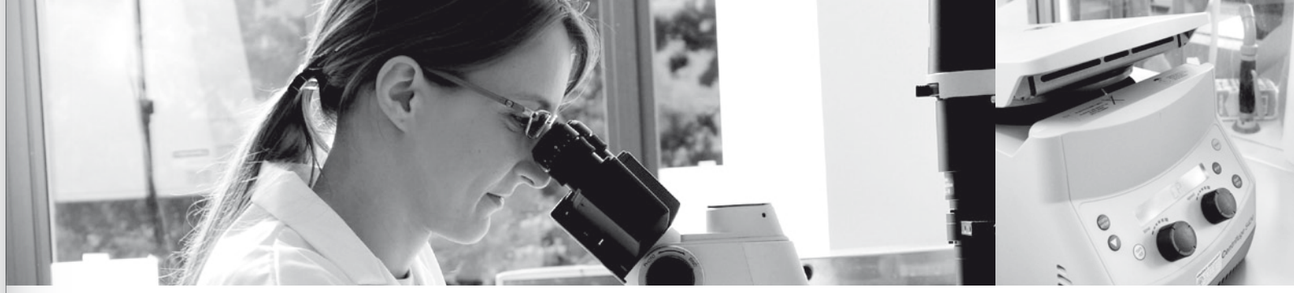
Federica Sallusto received her degree in Biology at the University of Rome La Sapienza. Between 1989 and 1996 she worked at the Department of Immunology, of the Italian National Institute of Health first as a postdoctoral fellow and then as a research scientist.

She worked at the Basel Institute for Immunology as a visiting scientist from 1993 to 1994 an as a member from 1996 to 2000.

Her research is focused on dendritic cell biology, T cell activation, differentiation and T cell traffic. Among her original contributions are the development of a method to culture human dendritic cells, the discovery that Th1, Th2 and Th17 cells express distinct sets of chemokine receptors and the definition of central and effector memory T cell subsets.

She published more than 80 papers and was the recepient of the Pharmacia Allergy Research Foundation Award in 1999.

Since 2000 Federica Sallusto is the Head of the Cellular Immunology Laboratory at the IRB.



Cellular Immunology

Specific immune responses require the timely interaction of various cell types within specific microenvironments. In the primary response the rare antigen specific naive T cells need to maximize the possibility of encounter with antigen. They do so by continuously recirculating through secondary lymphoid organs where they are stimulated by antigen-presenting mature dendritic cells (DCs). Soluble antigens can reach the lymph node directly but in most cases they are carried by migrating DCs that capture antigen in peripheral tissues and subsequently move through the lymphatics to the draining lymph node.

One goal of our laboratory is to understand how the number, localization and activation state of DCs in lymph node impact on T cell priming and immune responses.

A second goal of our research is to dissect the signals by which DCs determine differentiation of proliferating T cells towards the Th1, Th2 or Th17 lineage and how migratory capacity and effector function are coordinately regulated in differentiating T cells. Based on their migratory capacity and effector function we have originally characterized two subsets of memory T cells: central memory T cells (T_{CM}) express homing receptors for lymph nodes and have no or low level effector function. In contrast effector memory T cells (T_{EM}) lack lymph node receptors and have immediate effector function.

We are investigating the molecular basis underlying the functional properties and the differentiation potential of T_{CM} and T_{EM}, their heterogeneity and the signals required for their generation and maintenance. We are also interested to define the composition of memory subsets in different pathological and physiological conditions to gain insights into the role these subsets play in the immune responses.

Laboratory

Group Leader: Federica Sallusto, *PhD, 2000.*

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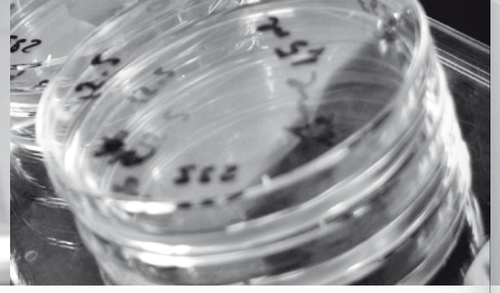


A cyanobacterial LPS antagonist prevents endotoxin shock and blocks sustained TLR4 stimulation required for cytokine expression.

Annalisa Macagno and Federica Sallusto.

Toll-like receptors (TLRs) function as primary sensors that elicit coordinated innate immune defenses through the recognition of microbial products and the induction of immune and pro-inflammatory genes. We identified and characterized a lipopolysaccharide (LPS)-like molecule extracted from the Cyanobacterium *Oscillatoria Planktothrix FP1* (CyP) that acts as a potent and selective antagonist of bacterial LPS. CyP did not induce any detectable response in human dendritic cells (DCs) and competitively inhibited LPS binding to the TLR4-MD-2 receptor complex. Addition of CyP together with LPS completely inhibited both MyD88- and TRIF-dependent pathways and suppressed the whole LPS-induced gene transcription program. CyP was effective in protecting mice from endotoxin shock in spite of a lower capacity to inhibit LPS stimulation of mouse DCs. Interestingly, delayed addition of CyP to DCs responding to LPS strongly inhibited signaling and cytokine production by immediate down-regulation of inflammatory cytokine mRNAs, while not affecting other aspects of DC maturation such as expression of MHC, costimulatory molecules and CCR7. Taken together these results indicate that CyP is a potent competitive inhibitor of LPS *in vitro* and *in vivo* and reveal the requirement of sustained TLR4 stimulation for induction of cytokine genes in human DCs. Current research aims at defining the molecular structure of CyP and to extend the characterization of its biological activity in different models of bacterial infection and inflammation. This work is done in collaboration with Carlo Rossetti, University of Insubria, Varese, Italy, Francesco Bertoni, Oncology Institute of Southern Switzerland (IOSI), Bellinzona, Swtizerland, Germain Puzo, Institute of Pharmacology and Structural Biology, CNRS, Toulouse, France, and Myron Christodoulides, School of Medicine, University of Southampton, UK.

• Macagno A. et al. / *J Exp Med 2006; 203:1481-1492* • Macagno A. et al. / *Trends Immunol 2007; 28:227-233*

Early and late primed T cells and the generation of immunological memory.

Miroslav Hons, Alfonso Martín-Fontecha and Federica Sallusto.

To investigate how the kinetics of immune response and dendritic cell (DC) activation impacts on T cell priming and the generation of effector and memory T cells *in vivo*, we set up a system where TCR transgenic $CD4^+$ T cells are activated in the early phases of an immune response by antigen displayed on highly stimulatory DCs ("active" DCs) or at later time points by antigen displayed on "exhausted" DCs. The results obtained so far indicate that early primed T cells divide extensively and efficiently differentiate to $CD62L^-$ effector cells producing IFN-γ. In contrast, late primed T cells perform only a few cell divisions, rapidly undergo reduction in cell size and stop proliferating. These cells remain $CD62L^+$ and do not acquire effector function, thus resembling T_{CM} cells. Upon secondary antigenic stimulation *in vivo*, late primed T cells respond significantly better than early primed T cells. These data suggest that *in vivo* the quality of effector and memory T cells is influenced by the kinetics of immune response and suggest one plausible mechanism for the generation of T_{CM} and T_{EM} cells.

L-selectin$^-$ CCR7$^-$ effector and memory CD8$^+$ T cells enter into reactive lymph nodes and kill dendritic cells.

Greta Guarda, Miroslav Hons, Alfonso Martín-Fontecha, and Federica Sallusto.

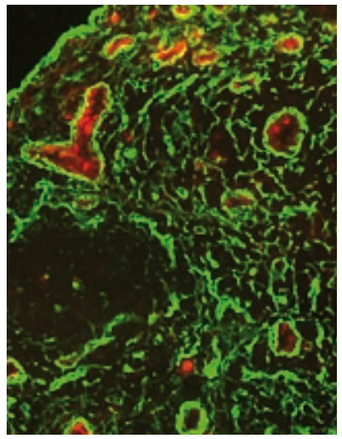


Figure 1: *CXCL9 (in red) on inflamed lymph no-de HEV surrounded by fibers of the fibroblastic reticular cell network (in green).*

T lymphocytes lacking the lymph node homing receptors L-selectin and CCR7 do not migrate to lymph nodes in the steady state. In contrast we found that lymph nodes that drain sites of mature dendritic cells (DCs) or adjuvant inoculation recruited L-selectin$^-$CCR7$^-$ effector and memory $CD8^+$ T cells. This cell recruitment required CXCR3 expression on T cells and occurred through high endothelial venules (HEVs) in concert with HEV luminal expression of the CXCR3 ligand CXCL9. In reactive lymph nodes, recruited T cells established stable interactions with and killed antigen-bearing DCs, limiting the ability of these DCs to activate naive $CD4^+$ and $CD8^+$ T cells. The inducible recruitment of blood-borne effector and memory T cells to lymph nodes may represent a mechanism for terminating primary and limiting secondary immune responses. This work was done in collaboration with Jens V. Stein, Theodor Kocher Institute, University of Bern, Bern, Switzerland and Ronald N. Germain, National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, MD, USA.

• Guarda G. et al. / *Nat Immunol 2007; 8:743-752*

Dendritic cell licencing and induction of autoimmunity by polyclonal effector memory T cells trafficking into chronic inflamed lymph nodes.

Alfonso Martín-Fontecha, Miroslav Hons, Greta Guarda, and Federica Sallusto.

We reported in previous studies that acutely stimulated lymph nodes undergo major changes in adhesive properties that result in the transient recruitment of NK cells and effector $CD8^+$ T cells. We now found that chronic inflammation of lym-ph nodes (induced by repeated dendritic cell (DC) injection or by some adjuvants) results in a long lasting expression of P-selectin on high endothelial venules (HEVs) and sustained recruitment of effector $CD62L^-$ $CD4^+$ T cells. Effector $CD4^+$ T cells in inflamed lymph nodes have a basal surface expression and preformed cytoplasmic CD154 that can trigger DC maturation in a CD40-dependent manner. We provide further evidence that effector $CD4^+$ T cells promote priming of naïve, antigen unrelated $CD4^+$ T cells *in vivo* in the absence of adjuvants. Importantly, we show that non pathogenic effector $CD4^+$ T cells mediate by-stander activation of autoreactive T cells that in turn trigger experimental autoimmune encephalomyelitis (EAE). Thus, tissue homing effector $CD4^+$ T cells can be redirected to chronically activated lymph nodes with unwanted consequences in the priming of antigen unrelated $CD4^+$ T cells.

Cell migration in antigen-stimulated lymph nodes and the control of adaptive immune responses.

Andrea Reboldi, Greta Guarda, Mariagrazia Uguccioni and Federica Sallusto.

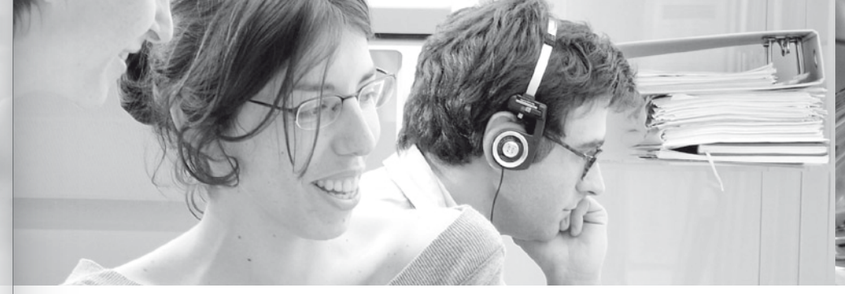
Naive T cell activation in lymph node is dependent on productive interactions with antigen-presenting stimulatory dendritic cells (DCs) that are migrating from peripheral tissues to draining lymph nodes. Frequency and duration of DC-T cell contacts and type of molecular interactions involved are key parameters in determining immune responses, ranging from tolerance to immunity. We previously show that inflammation increases migration to lymph nodes of DCs from tissues via afferent lymph and of T cells from blood via high endothelial venules (HEVs). More recently we found that L-selectin$^-$ CCR7$^-$ effector and memory cytotoxic $CD8^+$ T cells can migrate into inflamed lymph nodes. This cell recruitment requires CXCR3 expression on T cells and occurs through HEVs in concert with HEV luminal expression of the CXCR3 ligand CXCL9. In reactive lymph nodes, recruited T cells establish stable interactions with and kill antigen-bearing DCs, limiting the ability of these DCs to activate naive $CD4^+$ and $CD8^+$ T cells. By using *in situ* hybridization we are currently assessing whether CXCL9 is produced by HEVs and if other chemokines beside CXCL9 can be induced or downregulated in inflamed lymph nodes. We are also evaluating whether interfering with recruitment of effector and memory cytotoxic T cells to lymph nodes may increase primary and secondary immune responses through sustained antigen presentation by DCs.

Mouse IL-17-producing CD4$^+$ and CD8$^+$ T cells: differentiation and migration.

Andrea Reboldi, Eva V. Acosta-Rodriguez, Giorgio Napolitani and Federica Sallusto.

Effector function and migratory capacity are coordinately regulated during T cell differentiation. Th1 and Th2 cells express distinct sets of chemokine receptors that drive them to sites of DTH or allergic inflammation. Recently we found that human T cells differentiating *in vitro* into IL-17 producing T helper cells (Th17) up-regulate the chemokine receptors CCR6 and CCR4 and that human memory Th17 cells can be identified in blood and inflamed tissues by expression of CCR6 and CCR4. We are currently extending these findings to the mouse system. By using T cells from CCR6-deficient mice we are investigating the role of CCR6 in






directing migration of mouse Th17 cells in the steady state and in inflammatory conditions. We are also investigating the role of IL-1β in mouse Th17 differentiation in light of our findings that this cytokine plays a critical role in human Th17 differentiation (see below). This work is done in collaboration with Sergio A. Lira, Mount Sinai School of Medicine, New York, NY, USA.

Dissection of the B cell response in T cell-deficient mice

Dirk Baumjohann, Antonio Lanzavecchia, and Federica Sallusto.

We have set up an experimental system to study *in vivo* the requirements for induction of T cell-dependent B cell responses and for maintenance of memory B cells and serum antibody levels. We are using CD3γ$^{-/-}$ mice that lack T cells but exhibit normal B cell development and distribution in secondary lymphoid organs. In adoptive transfer experiments these mice are reconstituted with defined T cell populations from TCR-transgenic or allogeneic mice or from mice lacking certain chemokine receptors. To date we have established the assays needed for the readout of the system, i.e. characterization of lymphoid organ anatomy and B cell populations of CD3γ$^{-/-}$ mice by immunofluorescence microscopy and FACS analysis; assessment of serum immunoglobulin levels by ELISA; quantification of antibody secreting cells by ELISPOT. In a first series of experiments we found that upon immunization with a T cell-dependent model antigen (ovalbumin, OVA) in alum, CD3γ$^{-/-}$ mice were incapable of mounting an antigen-specific antibody response and that adoptive transfer of naïve OVA-specific TCR-transgenic T cells reconstituted the capacity of these mice to produce OVA-specific IgG antibodies.

However, these mice failed to mount secondary T cell and antibody responses. We are currently investigating the role of antigen and homeostatic signals in the induction of the unresponsive state. Furthermore we are optimizing this system to test different types of T cells (i.e. Th1, Th2, Th17, T_{FH}, T_{CM}, and T_{EM}) to define their impact on the humoral immune response.

Surface phenotype and antigenic specificity of human Th17 memory cells.

Eva V. Acosta-Rodriguez, Laura Rivino, Jens Geginat, David Jarrossay, Antonio Lanzavecchia, Federica Sallusto and Giorgio Napolitani

IL-17-producing T helper cells (Th17 cells) have been characterized in mice as a distinct subset of effector cells but their identity and properties in humans remain elusive. We found that CCR6 and CCR4 co-expression identifies human memory CD4$^+$ T cells that selectively produce IL-17 and express ROR-γt–encoding mRNA whereas CCR6 and CXCR3 identify IFN-γ producing Th1 cells and T cells that produce both IFN-γ and IL-17. Memory T cells specific for *Candida albicans* were found primarily in CCR6$^+$CCR4$^+$ Th17 subset whereas memory T cells specific for *Mycobacterium tuberculosis* were present in CCR6$^+$CXCR3$^+$ Th1 subset. Elicitation of IL-17 responses correlated with the capacity of *C. albicans* hyphae to stimulate antigen-presenting cells for priming of Th17 responses *in vitro* and for production of IL-23 but not IL-12. These results demonstrate that human Th17 have distinct migratory capacity and antigenic specificities and establish a link between microbial products, T helper cell differentiation and homing in response to fungal antigens.

• Acosta-Rodriguez E.V. et al. / *Nat Immunol 2007; 8:639-646*

Interleukins 1β and 6 but not transforming growth factor-β are essential for the differentiation of interleukin 17-producing human T helper cells.

Eva V. Acosta-Rodriguez, Giorgio Napolitani, Antonio Lanzavecchia and Federica Sallusto.

IL-17-producing CD4$^+$ helper T (Th17) cells have been implicated in host defense and autoimmune diseases. In the mouse, Th17 cell differentiation requires transforming growth factor-β (TGF-β) and IL-6 and the transcription factor RORγt. We found that in human naive CD4$^+$ T cells RORγt expression and Th17 polarization were induced by IL-1β and enhanced by IL-6, but suppressed by TGF-β and IL-12. Monocytes and conventional dendritic cells (DCs), but not monocyte-derived DCs activated by microbial stimuli efficiently induced Th17 priming and this function correlated with production by antigen presenting cells of IL-1β and IL-6 and not IL-12. These results identify cytokines, antigen-presenting cells and microbial products that promote human Th17 polarization and highlight an important difference in the requirements for Th17 differentiation in humans and mice.

• Acosta-Rodriguez E.V. et al. / *Nat Immunol 2007*

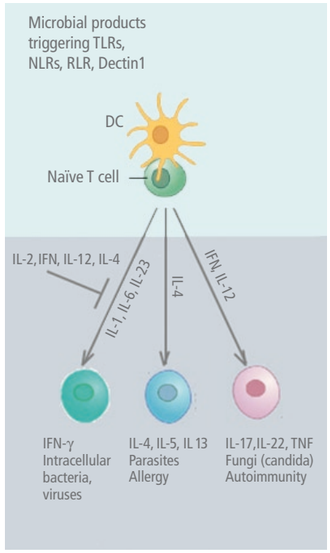


Figure 2: Differentiation pathways of human T helper cells.

The "T cell amplification method" as a new tool to analyze the repertoire of human naïve and memory T cells.

Rebekka A. Geiger, Thomas Duhen and Federica Sallusto.

By using T cell receptor (TCR) gene amplification and sequencing it was shown that circulating human naïve and memory T cell pools contain 25 x 10^6 and of 2 x 10^5 different TCR α chain sequences, respectively. Although useful to establish the heterogeneity of the T cell repertoires, these molecular approaches do not allow the establishment of a direct relationship between TCR and antigen-specificity. To investigate this question we developed a new method to overcome the major limitations for the analysis of the naïve and memory T cell repertoires – the low frequency of antigen specific cells and their high activation threshold.

We sorted CD4$^+$ T cell subsets from peripheral blood mononuclear cells (PBMC's) according to the expression of CD45RA and CCR7 and stimulated several replicates of limited numbers of naïve or memory T cells polyclonally with immobilized CD3 and CD28 antibodies or PHA + allogeneic PBMCs in the presence of IL-2. After a ~1000-5000 fold "cell amplification", each line contains 1-5 x 10^6 primed T cell blasts with increased sensitivity due to a higher number of responding cells and a low activation threshold. We identified cultures containing antigen-specific precursors by antigen-driven secondary stimulation using as read out ^{3}H-thymidine incorporation. This allows us to calculate frequency of antigen-specific T cells assuming that a polyclonal cell line contains one single antigen-specific precursor. Using this method we were able to assess the frequency of CD4$^+$T cel-ls specific for different antigens (such as primary antigens like KLH, Anthrax PA, Copaxone as well as recall antigens like TT, DerpI and PPD).

Surprisingly, our results show that frequencies of antigen-specific T cells in the naïve pool are much higher than previously thought whereas frequencies of antigen-specific T cells in the memory pool vary with the antigenic experience of the individual. We are also analyzing started the analysis of naïve and memory CD8$^+$ T cell repertoire against HIV and hCMV. Using HLA-A2 restricted peptides from CMV p65 and Gag, Env and Nef proteins we intend to generate hCMV- and HIV-specific CD8$^+$ T cell lines from hCMV-negative and hCMV-positive healthy individuals and from HIV-positive long-term non-progressor patients.

Effects of poxvirus vectors on dendritic cells and T cell responses.

Thomas Duhen, Antonio Lanzavecchia and Federica Sallusto.

In recent years, adenoviruses have become the favored vector to induce T cell responses, particularly by CD8 T cells. However, vector immunity remains a major issue for adenovirus vectors, whereas some poxvirus vectors have less of a problem. The PTVDC is a consortium of academic and industrial scientists, with expertise in poxvirus biology, innate and adaptive immunity and clinical development that aims to develop poxvirus vectors that maintain the safety profile established for existing highly-attenuated vectors, but that have an enhanced ability to elicit HIV-specific cellular immune responses.

In order to analyze existing unmodified poxvirus vectors with regard to their effect on innate immunity, we exposed monocyte-derived DCs (mo-DCs) to MVA and NYVAC viruses (wt and –C). We observed that only MVA was able to induce DC ma-turation (measured as CD86 and CD40 up-regulation), although it was less potent than a conventional maturation stimulus (LPS). No differences could be detected in induction of DC apoptosis. We are now planning to use these viruses to infect myeloid and plasmacytoid DCs purified from peripheral blood in order to prepare RNA samples for gene expression analysis using the Illumina platform.




Details

In order to develop assays to assess T cell stimulatory capacity of DCs exposed to parental and NYVAC and MVA mutants, we set up *in vitro* T cell priming assays using myeloid and plasmacytoid DCs in an allogeneic system. In these assays, DCs were activated with TLR ligands and the primed allogeneic T cell lines were analyzed for cytokine production and phenotype. Activation of naive CD4+ T cells with allogeneic myeloid DCs resulted in proliferation and secretion of IL-2, IL-4 and IFN-γ. Addition to the coculture of autologous plasmacytoid DCs (activated or not with CpG DNA) slightly increased T cell proliferation and augmented IFN-γ production and proportion of IFN-γ secreting T cells. The next step will be to determine the effect of MVA and NYVAC viruses on the different DC subsets and on the outcome of the T cell response. This work is done in collaboration with Rafick-Pierre Sekaly, University of Montreal-Saint-Luc, Montreal, Canada and the PTVDC consortium.

Isolation and characterization of human monoclonal antibodies against Dengue viruses.

Martina Beltramello, Antonio Lanzavecchia and Federica Sallusto.

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are caused by one of four closely related viruses (DENV1-4) of the genus *Flavivirus*. A first infection with one serotype induces a life-long protective immunity to the homologous serotype but no cross-protection against infection by a different serotype. Indeed, pre-existing immunity against a different serotype is associated with increased risk for DHF due to antibody-dependent enhancement (ADE). We are using an improved method of B cells immortalization with Epstein Barr Virus to isolate human DENV-specific antibodies (Abs) from memory B cells of donors recovered from primary DENV infection. Culture supernatants from po-lyclonal B cell lines containing DENV-specific Abs are identified by staining and FACS analysis of C6/36 mosquito cells infected with DENV. Specific Abs are also screened for their capacity to neutralize DENV infection of VERO cells by an in house developed FACS based assay. We identified several antibodies cross-reactive against DENV of different serotypes that showed significant neutralization activity. Further studies were performed to investigate enhancing activity on K562 cells that express Fc-receptor. All neutralizing Abs were found to enhance virus entry in a concentration-dependent way. In addition, some Abs showed enhancing but not neutralizing activity. Studies are ongoing to determine the specificity of the selected Abs. This work is done in collaboration with Cameron P. Simmons, Hospital for Tropical Diseases, Ho Chi Minh City, VietNam and Michael S. Diamond, Washington University School of Medicine, St. Louis, MO, USA.

Funding

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Collaborations

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Marco Gattorno / Institute G. Gaslini, Genoa, Italy.

Ronald N. Germain, Alex Y. Huang and Rosalind Polley / National Institute of Allergy and Infectious Diseases, National Institute of Health, Bethesda, MD, USA.

Sergio A. Lira / Mount Sinai School of Medicine, New York, NY, USA.

Germain Puzo / Institute of Pharmacology and Structural Biology, CNRS, Toulouse, France. Carlo Rossetti and Monica Molteni, University of Varese, Italy.

Rafick-Pierre Sekaly / University of Montreal-Saint-Luc, Montreal, Canada.

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Alessia Peter / Zurich University of Applied Sciences, Winterthur, Switzerland.

Elia Cattani / Scuola Superiore Medico Tecnica, Locarno, Switzerland.

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Seminar: "Cascades of DC and T-lymphocyte trafficking regulated by cognate interactions and chemokines."
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19.09.2005

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4.11.2005

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Karolinska Immunology Retreat, "Cell traffic regulation in the immune response".
Rosenön / *10-11.03.2006*

European Association for the Study of the Liver (EASL), 41st Annual Meeting, "The lesson from basic science: the role of adaptive immunity in viral infections"
Vienna / *26-30.04.2006*

MAIN Network of Excellence, Students' Meeting "Deciphering the Cell Migration Code", "Leukocyte migration in immune stimulated lymph nodes."
Gwatt / *13-16.05.2006*

Seminar: NIH/NIAID, Twinbrook Seminar Series, "Regulation of dendritic cell functivon by microbial products."
Rockville / *23.05.2006*

Pediatric Dengue Vaccine Initiative, Third Research Network Meeting, Arlie Center, "Isolation and characterization of human monoclonal antibodies against Dengue viruses."
Warrenton / *1-4.06.2006*

XXV EAACI Congress, "Chemoattractants and their receptors in inflammation."
Vienna / *10-14.06.2006*

DC-THERA Imaging Platform Course: In vivo dendritic cell migration, Nijmegen Centre for Molecular Life Sciences. "Dendritic cell and lymphocyte migration in lymph nodes."
Nijmegen / *10-14.07.2006*

The 2006 World Transplant Congress, "The link between innate and adaptive immunity."
Boston / *22-27.07.2006*

Cytokines 2006, Joint Conference of ICS, ISICR and ECS, "The impact of cytokine and chemokine networks on T cell priming."
Vienna / *27-31.08.2006*

First European Congress of Immunology, "Memory T cell subsets."
Paris / *6-10.09.2006*

International Graduate school in Molecular Medicine and International PhD Program in Cell and Molecular Biology, Lecture Course "Signal Transduction in T and B cell activation, development and differentiation", "Memory T cell origin and maintenance."
Milan / *18-22.09. 2006*

Seminar: "Cell traffic in lymph nodes during immune responses."
University of Bern, Bern Immunology Club seminar series / *27.09.2006*

Collaborative Research Centre 621 (SFB 621 "Pathology of the Intestinal Mucosa") Symposium, "Leukocyte traffic to the inflamed lymph node."
Hannover / *28-30.09.2006*

GSK Bio, Third Extramural R&D Symposium, "Understanding the generation and function of memory T cells."
Louvain-la-Neuve / *9-10.10.2006*

32nd Annual La Jolla Immunology Conference, "Memory T cell subsets: function and trafficking."
La Jolla / *10-12.10.2006*

Ernst Shering Foundation Symposium "Immunotherapy in 2020","Vaccination."
Postdam. / *22-24.10.2006.*

2007

Keystone Symposium "Immunological intervention in Human disease", Co-organizer (with Jacques Banchereau and Robert Coffman).
Big Sky Montana / *6-11.01.2007*

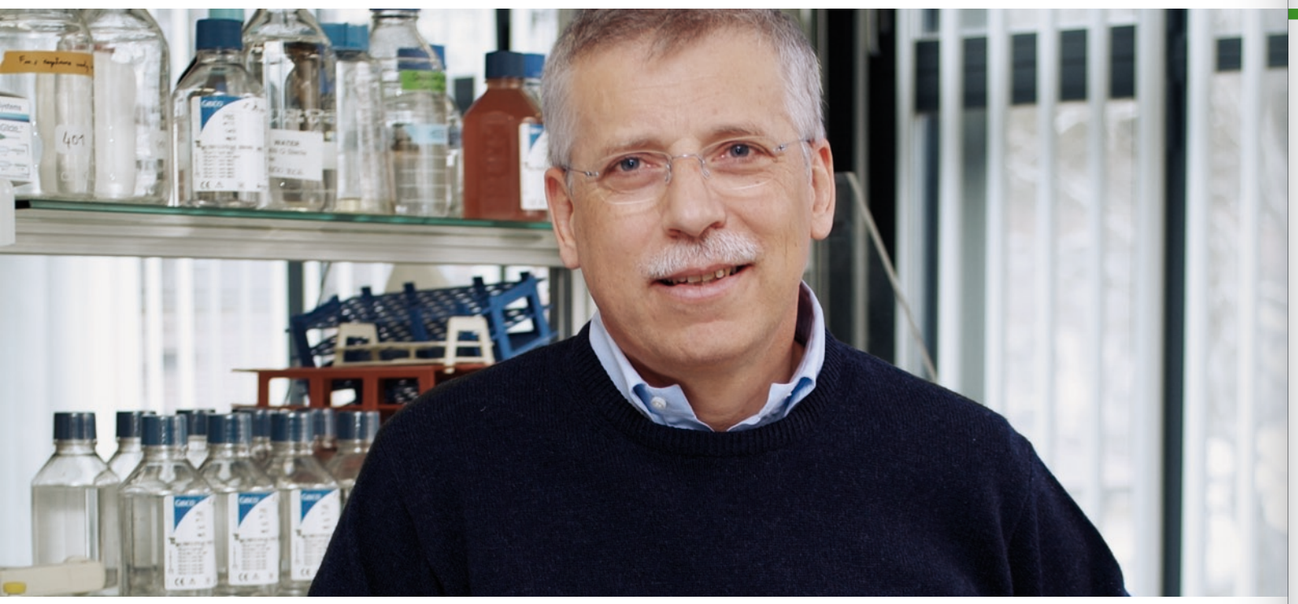
Keystone Symposium "Immunological intervention in Human disease", "T cell subsets in disease."
Big Sky Montana / *6-11.01.2007*

5th EAACI-GA2LEN Meeting "Basic Immunology Research in Allergy and Asthma", "Bridging innate and adaptive immunity"
Davos / *1-4.02.2007*

Keystone Symposium "The potent new anti-tunor immunotherapies", "Understanding the generation and function of memory T cell subsets."
Banff / *28.03-2.04.2007*

World Immune Regulation Meeting,"Distinction of effector and regulatory T cells in inflamed organs."
Davos / *11-15.04.2007*

ANALYTIC VACCINOLOGY



Antonio Lanzavecchia earned a degree in Medicine at the University of Pavia where he specialized in Paediatrics and in Infectious Diseases.

From 1983 to 1999 he was a member of the Basel Institute for Immunology and since 1999 he is the founding director of the Institute for Research in Biomedicine in Bellinzona. He has been Professor of Immunology at the University of Genoa and at the University of Siena.

Awarded the EMBO medal in 1988 and the Cloëtta prize in 1999, Dr. Lanzavecchia has published more than 200 papers.

His research has covered several aspects of human immunology: antigen processing and presentation, dendritic cell biology, lymphocyte activation and traffic, T and B cell memory.

Recently he developed a method for the efficient isolation of human monoclonal antibodies from memory B cells, which has been successfully applied to infectious diseases such as SARSCoV, H5N1, HCMV, Dengue, Malaria and HIV-1.

Immune Regulation

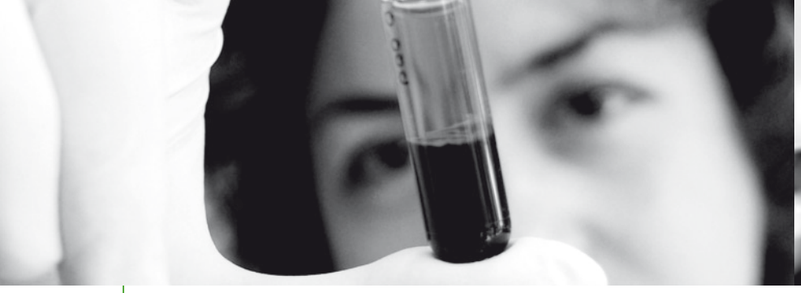
The research of our group remains focused on three main themes. First, we study the impact of innate immunity on the adaptive immune response with special emphasis on the activation of dendritic cell and the regulation of polarizing cytokines such as IL-12, IL-23, IL-1 and IL-6. Second, we continue to test in different experimental systems the role of the cumulative strength of stimulation (SoS) on the generation of effector and memory T cells. Current results from our as well as other groups support our initial proposition that SoS is the critical factor in determining the extent of CD4 and CD8 T cell differentiation. We are particularly interested to understand the mechanisms that control the generation of T and B memory cells and the dynamics of memory cells in the central and effector compartment. A third new avenue of research which is progressively expanding is prompted by two methods that have been originally developed in our laboratory that allow an accurate analysis of memory B cell frequencies and the efficient retrieval of human monoclonal antibodies from cells obtained from immune donors.

We feel that our research has the potential to impact in the field of vaccination at least in three areas: i) development of novel adjuvants capable of driving strong and selected immune responses; ii) identification of *in vitro* correlates of the immune status to evaluate vaccine efficacy and iii) adoptive immunotherapies with antigen-specific T cells or human monoclonal antibodies retrieved from the memory repertoire.

Laboratory

Group Leader: Antonio Lanzavecchia, *MD, 2000.*

Members: Afonso Almeida, *PhD, 2005* • Nadia Bernasconi, *PhD,2000* • Davide Corti, *PhD student,2004* • Giulia Di Lullo, *PhD, 2005* • Jens Geginat, *PhD, 2000-2006* • Isabella Giacchetto, *Technician, 2003* • Greta Guarda, *PhD student, 2004-2007* • David Jarrossay, *PhD, 2000* • Laura Lozza, *PhD student, 2005-2006* • Giorgio Napolitani, *PhD, 2002* • Debora Pinna, *PhD student, 2005* • Laura Rivino, *PhD student, 2003-2006* • Claudia Ruprecht, *PhD student, 2002-2006* • Chiara Silacci, *Technician, 2005* • Janine Stubbs, *PhD, 2006* • Fabrizia Vanzetta, *Technician, 2007* • Stefan Wirths, *MD, 2002-2006.*





TLR synergy controls a T helper type 1-polarizing program in dendritic cells.

Giorgio Napolitani, Federica Sallusto and Antonio Lanzavecchia.

Toll-like receptors (TLRs) sense microbial products and initiate adaptive immune responses by activating dendritic cells (DCs). As pathogens may contain several TLR agonists, we sought to determine whether different TLRs cooperate in DC activation.

In human and mouse DCs, TLR3 and TLR4 potently synergized with TLR7, TLR8 and TLR9 in the induction of a selected set of genes. Synergic TLR stimulation increased production of IL-12 and IL-23 and increased the Delta-4/Jagged-1 ratio, leading to DCs with enhanced and sustained Th 1-polarizing capacity. Global gene transcriptional analysis showed that TLR synergy boosted only 1% of the transcripts induced by single TLR agonists. These results identify a combinatorial code by which DCs discriminate pathogens and suggest new strategies for promoting T helper type 1 responses. This work was done in collaboration with Francesco Bertoni and Andrea Rinaldi, IOSI, Bellinzona, Switzerland.

• Napolitani et al. / *Nat Immunol 2005. 6: 769.*

• Macagno et al. / *Trends Immunol 2007. 28:227-233.*

Toll-like receptor stimulation provides a third signal for activation of human naive B cells.

Claudia Ruprecht and Antonio Lanzavecchia.

According to the current model, naive B cell activation is dependent on the sequential integration of two signals: B cell receptor (BCR) cross-linking by antigen, followed by cognate interaction with helper T cells through an immunological synapse. Using an improved method to purify human naive B cells we found that BCR stimulation and T cell help induced initial cell division but were not sufficient to promote survival and differentiation thus leading to abortive proliferation of naive B cells. Extensive B cell proliferation, isotypic switch and differentiation to immunoglobulin (Ig)-secreting cells was induced by addition of microbial products that trigger any of the Toll-like receptors (TLR) that are up-regulated in naive B cells upon BCR triggering. TLR agonists acted directly on B cells and were required irrespective of the nature of the T helper cells present. Supernatants of dendritic cells (DC) stimulated by DC-specific TLR agonists were also capable of enhancing B cell responses although to a much lower and variable extent. These results indicate that human naive B cell activation is critically dependent on innate stimuli acting optimally on TLR expressed by B cells. The coupling of BCR stimulation to TLR expression endows the human system with a high degree of specificity since it allows focusing of innate signals only on antigen-stimulated B cells.

• Ruprecht and Lanzavecchia, / *Eur. J. Immunol. 2006. 36: 810–816.*

Plasmacytoid DCs: accessory cells for germinal centre reactions.

David Jarrossay and Antonio Lanzavecchia.

CXCL13 is a chemokine produced in B cell areas that attracts $CXCR5^+$ cells such as resting B cells, follicular helper T cells (T_{FH}) present in B cell follicles and a small subset of central memory (T_{CM}) T cells. We found that plasmacytoid DCs stimulated by activated T cells upregulate CXCR5 and induce $CXCR5^+$ T_{CM} to differentiate to a T_{FH} like phenotype. In these conditions $CXCR5^+$ T_{CM} lose their capacity to migrate towards CCL21 but not towards CXCL13 and up-regulate CD57 (in a type I Interferon dependent fashion) and produce high levels of IL-10 (in an ICOS-L dependent fashion).
In a T-B cell coculture system pDCs induce B cells differentiation to plasmablasts, even without any TLR stimulation. We also found that in tonsils a fraction of pDCs express functional CXCR5, localize around germinal centers and are able together with T cells to induce B cell differentiation. These findings suggest that following inflammation and T cell priming, pDCs migrate to the inflamed lymph nodes or tonsil, upregulate CXCR5 and localize at the border of T cell areas where they can interact with $CXCR5^+$ T cells that enter the follicle and with antigen-activated B cells that exit the follicle. We therefore propose that pDCs may act as accessory cells in the development of the humoral response and the germinal centre reaction.

In mouse T cells the strength of stimulation (SoS) determines the extent of differentiation and memory generation.

Greta Guarda, Federica Sallusto and Antonio Lanzavecchia.

To investigate the requirements for ge-neration of effector and memory T cells, we set up an experimental system in which mouse TCR transgenic $CD4^+$ and $CD8^+$ T cells are stimulated *in vitro* by mature DCs and the strength of stimulation (SoS) delivered is varied as a function of the T/DC ratio and the duration of DC-T cell interaction. We found that L-selectin and CCR7 expression, as well as *in vivo* homing to the lymph nodes decreased progressively at increasing SoS, whereas responsiveness to homeostatic cytokines increased. Furthermore, the ability of the activated T cells to mount a secondary response *in vitro* and *in vivo* decreased as SoS increased. We conclude that, for both $CD4^+$ and $CD8^+$ T cells, memory generation requires an intermediate SoS, that is sufficient to make T cells responsive to homeostatic cytokines, while preserving their ability to home to secondary lymphoid organs and to respond to secondary antigenic challenge.

The strength of TCR stimulation determines IL-7 responsiveness, recall potential and lineage commitment of human CD4+IL-7Rhi T cells.

Laura Lozza, Laura Rivino, Davide Jarossay, Federica Sallusto, Antonio Lanzavecchia, and Jens Geginat.

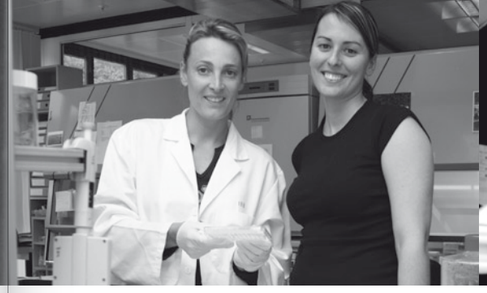
T-cell memory generation in the mouse requires IL-7 and memory precursors are IL-7Rhi, proliferate with homeostatic cytokines and expand upon antigenic re-stimulation. We analyzed how the strength of TCR stimulation regulates generation of human CD4+IL-7Rhi cells and their responses to IL-7 and TCR agonists. IL-7R α-_chain was rapidly lost under all priming conditions, but re-expression on proliferating cells was highest at an intermediate level of stimulation. CCR7-IL-7Rlo phenotype identified fragile effector cells, while CCR7+IL-7Rhi cells possessed cha-racteristics of memory precursors. Nevertheless, weak stimulation with immature DC generated CCR7+IL-7Rhi cells that responded poorly to IL-7. Conversely, strongly stimulated CCR7+IL-7Rhi cells expanded efficiently with IL-7 and differentiated spontaneously to Th1 effector cells. This high IL-7 responsiveness was associated with reduced PTEN expression levels and enhanced S6-kinase activation, but not with altered STAT5 phosphorylation. A strong stimulation impaired however IL-2 secretion and expansion upon TCR re-stimulation, while an intermediate level of stimulation allowed for optimal secondary expansion and slow proliferation with IL-7 that was not associated with differentiation. Gene expression analysis suggested that strongly stimulated CCR7+IL-7Rhi cells had a higher metabolic rate, corroborated Th1 lineage commitment and provided a plausible explanation for their low recall potential. Our results are consistent with the view that T-cell memory is generated at an intermediate range of signal strength and suggest that circulating human memory subsets could be derived from CCR7+ precursors that received different amounts of stimulation. This work was done in collaboration with Andrea Rinaldi and Francesco Bertoni, IOSI, Bellinzona, Switzerland.

Contribution of $CD8^+$ T cell subsets to the recovery of the peripheral $CD8^+$ T cell pool after lymphopenia or irradiation.

Afonso Almeida and Antonio Lanzavecchia.

The process of lymphopenia driven proliferation is a remarkable example of homeostatic regulation of the immune system and is dependent on both cytokines and TCR-MHC interactions.

These processes should also take part in the recovery of peripheral lymphocyte numbers following irradiation. Although irradiation protocols are relevant in therapy, it is still unknown to what extent secondary effects in the Immune system add to direct effects of irradiation for instance in facilitation of tumor recognition by the host's immune system. We have developed a transfer system using congenic T cells identified by allotypic markers (Ly5 and Thy.1 alleles) to address the *in vivo* expansion and reconstitution potential after transfer of T cell subsets into T cell deficient ($CD3e^{-/-}$) or irradiated hosts. We found that in both cases CD62L+ central memory $CD8^+$ T cells are the major contributors for the reconstituted pool. In contrast naïve T cells and CD62L- effector memory CD8+ T cells contribute little to the recovered pool in T cell deficient mice and only marginally in irradiated recipients. We also found that expanding central memory T cells generate a progeny with both central and effector memory phenotype, while effector memory cells show very little if any


phenotypic conversion. We are currently performing experiments with TCR transgenic T cells and evaluating the contribution of the reconstituted CD8 pool to the immunocompetence of the individual. These findings will be relevant for the designing of adoptive transfer therapies and for the prediction of their secondary effects. This work is done in collaboration with Dr. Ilja Ciernik, IOSI, Bellinzona

Modeling serological memory.

Chiara Borsotti, Markus Manz and Antonio Lanzavecchia.

Long-lived bone marrow resident plasma cells and memory B cells, stimulated to differentiate to plasma cells by persisting antigen or by polyclonal stimulation, may contribute to sustain serum antibody levels, eventually for a lifetime. However, their relative contribution to a continuous antibody production remains to be established. We monitored for two years the kinetics of circulating plasma cells, memory B cells and serum antibodies in 8 healthy volunteers that had been boosted with tetanus toxoid (TT). In all cases we observed a rapid elevation of serum antibodies (10-100 fold increase from day 6 to day 10) followed by a plateau lasting about a month, which was followed by a linear decrease in antibody levels until a stable plateau value was reached after 6-8 months. The experimental values obtained have been used to elaborate a mathematical model which is based on three parameters: i) the magnitude of the antigen driven short lived plasma cell response (responsible for the rapid elevation of serum antibodies); ii) the magnitude of the antigen driven long lived plasma cell response (that sustain serum antibodies for a few months) and iii) the extent of homeostatic proliferation and differentiation of memory B cells (that sustains antibody production after the antigen dependent phase). Based on these results and their modeling we propose two memory phases: a short term memory, which is determined by short lived and long lived plasma cells generated following antigenic stimulation and a long term memory that is maintained through antigen independent polyclonal activation of memory B cells. To test this model we are currently monitoring serum antibody levels in patients treated with the anti-CD20 antibody Rituximab. Since anti-CD20 antibody depletes B cells but not plasma cells we expect to measure *in vivo* the contribution of memory B cells to antibody production in the absence of antigenic stimulation. This work is done in collaboration with Elisabetta Traggiai, a former member of the laboratory and with Roberto Puzone, IST, Genova.

ACB1 transporter discriminates human resting naïve B cells from cycling transitional and memory B cells.

Stefan Wirths and Antonio Lanzavecchia.

The exact identification of B cell subsets is instrumental to understand their triggering requirements and their dynamics under physiological and pathological conditions. Human memory B cells are currently identified according to the expression of CD27, which is absent on naive B cells. We found that the ATP-binding cassette (ABC)B1 transporter is exclusively present on mature CD27– naive B cells, while it is absent in CD27+ memory B cells and in a heterogeneous subset of CD27– cells that comprise both switch memory and transitional B cells. Thus, ABCB1 activity precisely discriminates naive from transitional and all memory B cells. Using this improved method to discriminate human B cell subsets, and Ki67 staining to identify recently divided cells, we found that in both cord blood and adult peripheral blood, mature naive B cells are quiescent while transitional B cells and memory B cells have a high *in vivo* turnover.

• Wirtz and Lanzavecchia, Yang et al *Eur. J. Immunol. 2005. 35: 3433-41.*

Human monoclonal antibodies that neutralize SARS-coronaviruses with high potency breadth.

Davide Corti and Antonio Lanzavecchia.

Using an improved method of EBV immortalization of human memory B cells we previously reported the isolation from a patient recovered from SARS of several monoclonal antibodies that neutralize both *in vitro* and *in vivo* the homologous SARS-CoV (Traggiai et al. Nat. Medicine 2004. These antibodies have been further characterized in terms of: i) potency, ii) breadth of reactivity on human and animal isolates and iii) epitope mapping. Three antibodies were capable of neutralizing with high potency both human and animal SARS-CoV isolates. The epitopes recognized by two of such antibodies were mapped by isolation of escape mutants, while no escape has been observed so far fro the third antibody. These results demonstrate that it is possible to select from a pool of specific antibodies those with superior performance in terms of breadth and potency of neutralization. This work was done in collaboration with Stephan Becker, University of Marburg, Kanta Subbarao and Gary Nabel, NIAID, Bethesda and Barry Rockx and Ralph Baric, University of North Carolina.

• Yang et al. / *PNAS 102: 2005. 797-801.*

• Voss et al / *FEBS letters 2006. 580:968-973.*

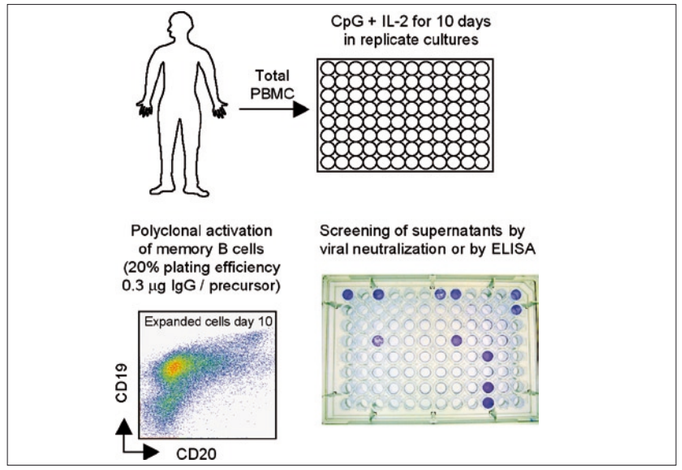
Antigen-specific memory B cell repertoire analysis: dissecting useful and junk specificities.

Debora Pinna, Davide Corti and Antonio Lanzavecchia.

In order to characterize the humoral immune response to infections or vaccination, we developed a quantitative method to interrogate the human antigen specific memory B cell repertoire.

Total PBMC are polyclonally stimulated in the presence of IL-2 and B cell Toll Like Receptors agonists for 10 days. This culture condition leads to a selective expansion of the B cell memory compartment and its differentiation into antibody secreting cells. The main advantage of this method is the possibility to interrogate the B cell repertoire at a clonal level. The frequency of antigen specific B cell precursors can be quantitatively determined even when they are rare. Furthermore it is possible to make several assays on single monoclonal products. As an example we characterize the specificity and the cross-reactivity of many B cells for different hemagglutinins of the Influenza A virus. We also show that for a given pathogen only a minor fraction of specific B cells had neutralizing activity while the majority is done against not neutralizing epitopes. This method should be useful for assessing the potency of vaccines and the clonal composition of B cell memory pool.

Figure 1: the Antigen-Specific Memory B cell Repertoir Analysis (AMBRA) method.



Neutralization of bacterial toxins by human monoclonal antibodies.

Davide Corti, Nadia Bernasconi, Antonio Lanzavecchia.

Horse antibodies to bacterial toxins have been used as life-saving drugs for more than a century. However there are still no human antibodies available to neutralize Diphteria or Anthrax toxins in exposed individuals. By immortalizing memory B cells from an immunized volunteer we were able to isolate several monoclonal antibodies that reacted in ELISA with Anthrax toxin protective antigen (PA). Only a fraction of the antibodies neutralized the Anthrax holotoxin (PA+LF) *in vitro*, while others failed to neutralize or showed a marked prozone. The most effective antibody (A146) completely neutralized Anthrax holotoxin at sub-stoichiometric concentrations both *in vitro* and in an animal challenge model. In another setting, individuals with high antibody titers to Diphteria toxin (DT) were identified and several monoclonal antibodies were isolated. The most effective antibody (D2.2) neutralized DT *in vitro* in a stoichiometric fashion at concentrations as low as 10^{-11}M. We have produced mammalian expression vectors to transfect CHO cells in order to produce these antibodies in a system suitable for human use. In collaboration with Luca Varani we will attempt to map the epitopes recognized by these antibodies using an in silico docking approach. This work was done in collaboration with Bob Mittler, Emory University and Cesare Montecucco, University of Padova.




Human pregnancy-associated malaria-specific B cells target polymorphic conformational epitopes in VAR2CSA.

Nadia Bernasconi and Antonio Lanzavecchia.

Pregnancy-associated malaria (PAM) is caused by the binding of *Plasmodium falciparum*-infected erythrocytes to chondroitin sulphate A (CSA) in the placenta mediated by PAM-associated clonally va-riant surface antigens which include the PfEMP1 variant VAR2CSA. We isolated from B cells of multiparous African women eight human monoclonal IgG1 antibodies that react exclusively with intact CSA-adhering IEs. Four reacted in Western blotting with high MW proteins, while seven reacted with either the DBL3-X or the DBL5-e domains of VAR2CSA expressed as *Baculovirus* constructs or on the surface of transfected Jurkat cells. We used a panel of recombinant antigens representing DBL3-X domains from *P. falciparum* field isolates to evaluate B-cell epitope diversity among parasite isolates, and identified the binding site of one monoclonal antibody using a chimeric DBL3-X construct. Our findings show that VAR2CSA is a primary target of naturally acquired PAM-specific protective immunity, and demonstrate the value of human monoclonal antibodies and conformationally intact recombinant antigens in VSA characterization. This work is done in collaboration with Lea Barfod and Lars Hviid, Rigshospitalet, Copenhagen.

• Barfod et al. / *Mol Microbiol 2007. 63: 335-47.*

Dissecting the human antibody response to Plasmodium falciparum.

Janine Stubbs and Antonio Lanzavecchia.

We aim to take a novel, unbiased approach to interrogate the memory B-cell repertoire of individuals immune to malaria to establish the frequency of memory B cells and isolate protective and possibly pathogenetic antibodies. The antibodies will be selected according to their ability to bind to and functionally inhibit blood-stage parasites in *in vitro* correlative tests of *in vivo* protection. The antigenic targets of human monoclonal antibodies will be determined by a variety of mole-cular methods. To further associate the identified host-parasite interactions with protection from malaria, immuno-epidemiological studies will be conducted in collaboration with Kevin Marsh, Kilifi, Kenya. In a preliminary series of experiments we found that a lerge fraction of memory B cells produce antibodies that stain hemozoin associated antigens that are present in the food vacuole. We are currently investigating whether such antibodies play a pathogenetic role in malaria infection.

Prophylactic and therapeutic efficacy of human monoclonal antibodies against H5N1 influenza.

Nadia Bernasconi and Antonio Lanzavecchia.

New prophylactic and therapeutic strategies to combat human infections with highly pathogenic avian influenza H5N1 viruses are needed.

We isolated H5N1 neutralizing monoclonal antibodies from Vietnamese adults who had recovered from infections with H5N1 viruses. The cross-reactivity of these antibodies for different strains of H5N1 was tested *in vitro* by neutralization assays, and their prophylactic and therapeutic efficacy *in vivo* was tested in mice. *In vitro*, mAbs FLA3.14 and FLD20.19 neutralized both Clade I and Clade II H5N1 viruses, whilst FLA5.10 and FLD21.140 neutralized Clade I viruses only. *In vivo*, FLA3.14 and FLA5.10 conferred protection from lethality in mice challenged with A/Vietnam/1203/04 (H5N1) in a dose-dependent manner. mAb prophylaxis provided a statistically significant reduction in pulmonary virus titer, reduced associated inflammation in the lungs, and restricted extrapulmonary dissemination of the virus. Therapeutic doses of FLA3.14, FLA5.10, FLD20.19, and FLD21.140 provided robust protection from lethality at least up to 72 h postinfection with A/Vietnam/1203/04 (H5N1). mAbs FLA3.14, FLD21.140 and FLD20.19, but not FLA5.10, were also therapeutically active *in vivo* against the Clade II virus A/Indonesia/5/2005 (H5N1). These studies provide proof of concept that fully human mAbs with neutralizing activity can be rapidly generated from the peripheral blood of convalescent patients and that these mAbs are effective for the prevention and treatment of H5N1 infection in a mouse model. A panel of neutralizing, cross-reactive mAbs might be useful for prophylaxis or adjunctive treatment of human cases of H5N1 influenza. This work was done in collaboration wit Cameron Simmons, Hospital for tropical diseases, Vietnam and Kanta Subbarao, NIAID, USA.

• Simmons et al. / *PLOS Medicine 2007 4:e178.*

Isolation of human antibodies that neutralize non clade B HIV-1.

Davide Corti, Debora Pinna and Antonio Lanzavecchia.

In more than 20 years of attempts only a few monoclonal antibodies have been isolated that are capable of broadly neutralizing HIV-1 isolated, primarily from clade B. The analysis of the epitopes recognized by such antibodies is instrumental to designing of new immunogens capable of eliciting such broad neutralising antibody responses. We are using the efficient immortalization method to isolate neutralizing antibodies from non clade B patients. The supernatants of immortalized cells are screened to detect antibodies that either bind non clade B gp140 trimers or neutralize non clade B HIV-1 pseudotypes. Although the frequency of memory B cells specific for HIV-1 antigens is generally low, we have been able to isolate several monoclonal antibodies that potently neutralize different clade C and clade A viruses. This work is done in the framework of the VDAC consortium coordinated by Prof. Robin Weiss, UCL London.

Attempts to identify the cellular basis of IgE memory.

Giulia Di Lullo and Antonio Lanzavecchia.

Memory B cells carrying surface IgG or IgA antibodies can be readily isolated and immortalized. However it is not clear whether IgE+ memory B cells may also exist, a fact that is relevant to understand memory IgE responses. Using the AMBRA method as well as enhanced EBV immortalization we set up to identify human memory B cells capable of secreting IgE antibodies from allergic individuals. The possibility that IgE+ switch memory cells might exist was ruled out by sorting experiments that failed to identify an IgE+ population capable of producing IgE *in vitro* upon expansion of immortalization. We therefore hypothesized that allergen specific memory cells may carry a different isotype and may switch to IgE only when they terminally differentiate. However this mechanism is unlikely since a large fraction of memory B cells is capable of undergoing secondary switch to IgE if stimulated in the presence of IL-4. We are therefore considering a third hypothesis namely that allergen specific memory B cells are mIgE- and have a unique capacity to home to allergen exposed sites where they undergo class switch recombination and terminal differentiation in response to local cytokines including IL-4. Indeed, immortalization of IgG+ memory B cells in the presence of IL-4 led to the isolation of several B cell clones that express membrane IgE and secrete IgE in the culture supernatant at concentrations ranging from 10 to 40 µg/ml. Alternatively we envisage that most memory B cells home to allergen exposed sites but only the allergen specific B cells can be triggered by allergen-specific Th2 cells to switch and differentiate to IgE-secreting plasma cells (specificity of the IgE response due to cognate Th2 help). Experiments are underway to correlate the expression of homing receptors on memory B cell subsets that contain allergen specific memory B cells.

Autoreactive B cells in Pemphigus vulgaris: isolation of monoclonal antibodies and effect of anti-CD20 treatment.

Giulia Di Lullo and Antonio Lanzavecchia.

Pemphigus vulgaris (PV) is a severe bullous skin disease caused by the production of pathogenetic antibodies to Desmogleins that can be effectively treated by administration of anti-CD20 antibodies. We isolated mononuclear cells from PV patients before and at different times after treatment with anti-CD20.

B cells from untreated donors were immortalized and several human monoclonal antibodies specific for desmogleins and keratinocyte antigens were isolated. We are characterizing such antibodies as to their fine specificity and function. We will then determine to what extent the anti-CD20 therapy will impact on autoreactive versus conventional memory B cells. This work is done in collaboration with Giovanni Di Zenzo and Giovanna Zambruno, IDI-IRCCS, Roma.

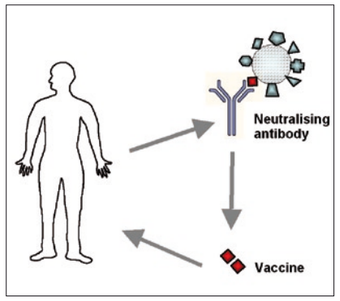
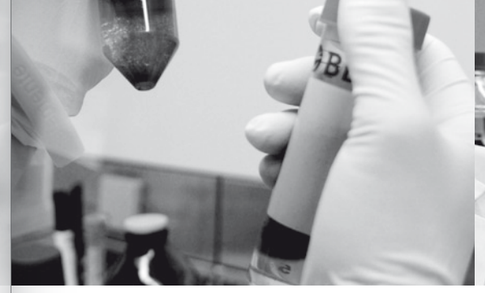


Figure 2: *The analytic vaccinology approach to antibody-based vaccines*





Details

Analytic vaccinology: the case of cytomegalovirus.

Annalisa Macagno, Nadia Bernasconi and Antonio Lanzavecchia.

Neutralizing antibodies can be used not only as therapeutics in a passive vaccination setting, but also as tools to identify critical molecular patterns of a pathogen that are required for infectivity.

Once identified and produced in an appropriate form, these structures should be able to elicit a neutralizing antibody response. This approach, that we define as "analytic vaccinology", can be effectively implemented using the memory B cell immortalization approach described above. We have explored the potential of this approach in the case of human cytomegalovirus (HCMV). Three types of HCMV-neutralizing IgG antibodies were isolated: i) antibodies that neutralize only infection of fibroblasts; ii) antibodies that neutralize the infection of both fibroblasts and endothelial cells and iii) antibodies that neutralize only infection of endothelial cells. The latter were extremely potent since neutralization was observed in the ng/ml range. By expressing candidate HCMV genes in HEK293T cells we mapped the specificities of such antibodies to the products of two genes, UL130 and UL131A. These findings identify critical epitopes in a molecular complex that is essential for HCMV infectivity of endothelial, epithelial and dendritic cells.

Funding

Swiss National Science Foundation, Dendritic cells: origin, maturation and impact on t cell responses.
3100A0-063885 / *2001-2006*

Swiss National Science Foundation, The role of innate receptors in dendritic cell and B cell activation.
3100A0-112678 / *2006-2009*

Swiss National Science Foundation (to Jens Geginat), Molecular mechanisms determining T cell fates.
3100A0-104168 / *2004-2007*

Vaccine induced immunity in the young and aged.
NIH: U19-AI057266-01 / *2003-2008*

European Community: MUVAPRED: Mucosal vaccines for poverty related diseases.
FP6-LSHG-CT-2003-503240, BBW-Nr. 03.0570-1, *2004-2008*

European Community: MAIN: Targeting cell migration in chronic inflammation.
FP6-NoE LSHG-CT-2003-502935, BBW-Nr. 03.0441-1 / *2003-2008*

European Community: SARSVAC: Immunoprevention and immunotherapy of SARS infection.
FP6-SP-CT-2004-511065 / *2004-2007*

European Community: DC-THERA: Dendritic cells for novel immunotherapies.
FP6 –NoE-LSHB-CT-2004-512074 / *2005-2009*

European Community: MUGEN: Murine models of human immunological disease.
FP6 –NoE-LSHG-CT-2005-005203 / *2005-2009*

European Community: HUMALMAB: Human monoclonal antibodies for Malaria research and therapy.
2007-2009

The Wellcome Trust: Generation and characterization of human monoclonal antibodies to highly pathogenic H5N1 viruses.
079864/Z/06/Z / *2006-2009*

Bill & Melinda Gates Foundation. VDAC: Vaccine induced protective crossneutralization of HIV-1.
2006-2011

Bill & Melinda Gates Foundation: PTVDC: Poxvirus T cell vaccine discovery consortium.
2006-2011

Collaborations

Pascal Poignard / CIML, Marseille, France

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Cesare Montecucco / University of Padova, Italy

Ilja Ciernik / IOSI, Bellinzona, Switzerland

Publications

2005

Dendritic cell-derived IL-2 production is regulated by IL-15 in humans and in mice.
Feau S., Facchinetti V., Granucci F., Citterio S., Jarrossay D., Seresini S., Protti M. P., Lanzavecchia A., and Ricciardi-Castagnoli P. / *Blood 2005; 105:697-702*

Evasion of antibody neutralization in emerging severe acute respiratory syndrome coronaviruses.
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Immune subversion by Helicobacter pylori
Baldari C. T., Lanzavecchia A., and Telford J. L.
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T cell costimulation by chemokine receptors.
Molon B., Gri G., Bettella M., Gomez-Mouton C., Lanzavecchia A., Martinez A., Manes S., and Viola A. / *Nat Immunol 2005; 6:465-471*

Understanding the generation and function. of memory T cell subsets
Lanzavecchia A. and Sallusto F. / *Curr Opin Immunol 2005; 17:326-332*

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Human Adaptive Immune System Rag2-/- {gamma}c-/- Mice.
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Napolitani G., Rinaldi A., Bertoni F., Sallusto F., and Lanzavecchia A. / *Nat Immunol 2005; 6:769-776*

ABCB1 transporter discriminates human resting naive B cells from cycling transitional and memory B cells.
Wirths S. and Lanzavecchia A. / *Eur J Immunol 2005; 35:3433-3441*

2006

Activation of the Flt3 signal transduction cascade rescues and enhances type I interferon-producing and dendritic cell development.
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Regulation of peripheral T cell activation by calreticulin.
Porcellini S., Traggiai E., Schenk U., Ferrera D., Matteoli M., Lanzavecchia A., Michalak M., and Grassi F. / *J Exp Med 2006; 203:461-471*

Toll-like receptor stimulation as a third signal required for activation of human naive B cells.
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Understanding and making use of human memory B cells.
Lanzavecchia A., Bernasconi N., Traggiai E., Ruprecht C. R., Corti D., and Sallusto F.
Immunol Rev 2006; 211:303-309

Simvastatin inhibits the MHC class II pathway of antigen presentation by impairing Ras superfamily GTPases.
Ghittoni R., Napolitani G., Benati D., Ulivieri C., Patrussi L., Laghi Pasini F., Lanzavecchia A., and Baldari C. T. / *Eur J Immunol 2006; 36:2885-2893*

2007

Distinct expression pattern of IFN-alpha and TNF-alpha in juvenile idiopathic arthritis synovial tissue.
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Human pregnancy-associated malaria-specific B cells target polymorphic, conformational epitopes in VAR2CSA.
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L-selectin-negative CCR7- effector and memory CD8+ T cells enter reactive lymph nodes and kill dendritic cells.
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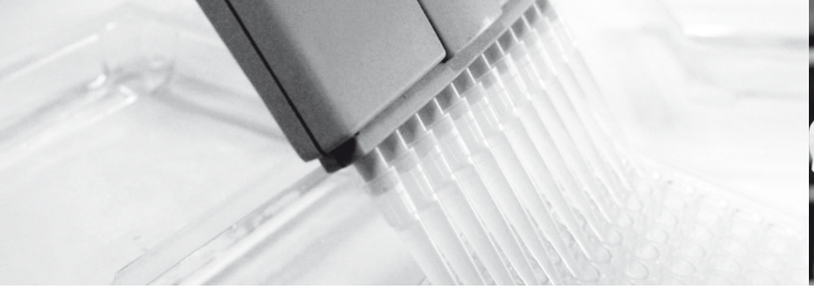
Nat Immunol 2007; 8:743-752

Prophylactic and therapeutic efficacy of human monoclonal antibodies against H5N1 influenza.
Simmons C. P., Bernasconi N. L., Suguitan A. L., Mills K., Ward J. M., Chau N.V., Hien T.T., Sallusto F., Ha do Q., Farrar J., de Jong, Lanzavecchia A., and Subbarao K. / *PLoS Med 2007; 4:e178*

Potent cross-reactive neutralization of SARS coronavirus isolates by human monoclonal antibodies.
Zhu Z., Chakraborti S., He Y., Roberts A., Sheahan T., Xiao X., Hensley L. E., Prabakaran P., Rockx B., Sidorov I. A., Corti D., Vogel L., Feng Y., Kim J.O., Wang L. F., Baric R., Lanzavecchia A., Curtis K. M., Nabel G. J., Subbarao K., Jiang S., and Dimitrov D. S. / *Proc Natl Acad Sci U S A 2007; 104:12123-12128*





Division of labor with a workforce of one: challenges in specifying effector and memory T cell fate.
Reiner S. L., Sallusto F., and Lanzavecchia A.
Science 2007; 317:622-625

Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells.
Acosta-Rodriguez E.V., Napolitani G., Lanzavecchia A., and Sallusto F. / *Nat Immunol 2007;*

Book Chapters

Sallusto F, Lanzavecchia A. The role of dendritic cells in T cell activation and differentiation. In: Handbook of dendritic cells: Biology, Diseases, and Therapies.
Lutz MB, Romani N, Steinkasserer A Eds, Wiley-VCH Weinhelm / *2006*

Lectures and Seminars

2005

The Henry Kunkel Society Annual Meeting "Infection, Inflammation and Autoimmunity": "Vaccination and immunological memory".
Cambridge, UK / *2005*

NIAID 2nd International Conference "Dendritic cells at the Host-Pathogen Interface": "Dissecting DC maturation programs".
Airlie, USA / *2005*

Biogen Idec Immunology Forum on Viral Pathogens, "The role of microbial products in dendritic cell and B cell activation."
Cambridge, MA, USA / *2005*

Immunoway, Immunologia Umana 2005, "Acquisizioni sulla memoria dei linfociti-B e possibili impieghi."
Ruta di Camogli, Italy / *2005*
Center for the Study of Inflammatory Bowel Disease, Boston, MA, USA."B Cell and Plasma Cell Memory".

American College of Rheumatology Annual scientific meeting, "From antigen presentation to immunological Memory."
San Diego, USA / *2005*

Seminar: "The role of TLRs in dendritic cell and B cell activation."
MD Anderson Cancer Center, Houston, USA
2005

Cours d'Immunologie approfondie, "Human naïve and memory B cells: identification, activation and use."
Institut Pasteur, Paris, France / *2005*

Seminar: "The role of Toll-like receptors in innate and adaptive immunity."
IRBM Merck, Roma, Italy / *2005*

Monoclonal Antibodies: Research, Development and Application, "Immunotherapy and Infectious Diseases."
Istituto Superiore di Sanità, Roma, Italy / *2005*

Annual Meeting Dutch Society for Immunology, "Cell traffic in the lymph node and control of T cell immunity."
Noordwijkerhout, The Netherlands / *2005*

Seminar at the Dept. of Pathology, "Human naïve and memory B lymphocytes: Identification, activation, homeostasis and exploitation."
University of Amsterdam, The Netherlands
2005

2006

Keynote address at Keystone Symposium: Determinants of Host Resistance, Susceptibility or Immunopathology to Pathogens. "The Immune Battle Against Infectious Organisms: From Mouse Models to Human Disease"
Keystone Resort, Keystone Colorado / *2006*

Keystone Symposium: Lymphocyte Activation and Signaling. "Signals that Drive Immunological Memory"
Keystone Resort, Keystone Colorado / *2006*

Corso di formazione, "Linfociti memoria e immunità a lungo termine."
Collegio Ghislieri, Pavia, Italia / *2006*

ZMBH colloquia series, "Human monoclonal antibodies and analytic vaccinology."
Heidelberg, Germany / *2006*

Serata di formazione per i farmacisti, "I vaccini del futuro."
Scuola Arti e Mestieri, Bellinzona, Switzerland
2006

Seminar: "Human naïve and memory B cells: identification, activation, and exploitation."
University of Alabama, Birmingham, USA
2006

Nomura Science Enterprise Lecture series. "Active and passive vaccination."
King's college, London, UK / *2006*

Seminar at CRUK, "On the cellular basis of immunological memory."
Lincoln's Inn Field Laboratories, London, UK
2006

Seminario IOSI, "Le basi cellulari della memoria immunologica."
Ospedale Regionale Bellinzona, Switzerland
2006

DC-Crest workshop:"Dendritic cell activation."
Celerina, Switzerland / *2006*

VRC Symposium: "Vaccination and immunological memory."
Natcher Building, NIAID, Bethesda, USA / *2006*

Keynote lecture at the Third Workshop SIICA, "Memoria B e anticorpi monoclonali."
Certosa di Pontignano, Siena, Italy / *2006*

Annual Meeting of The American Association of Immunologists, "B lymphocyte activation."
Boston, USA / *2006*

Lecture: "Naïve/memory CD4 T cells."
ENII-MUGEN Summer School, Sardegna, Italy
2006

Presentation of IRB research to Prof. Dr. H.R.H Princess Chulabhorn Mahidol.
IRB, Bellinzona / *2006*

Seminar: "Exploring and exploiting immunological memory."
Novartis Institute for Tropical Diseases, Singapore / *2006*

Keynote lecture, Gaslini advanced course in basic and applied immunology, "The cellular basis of immunological memory."
Genoa, Italy / *2006*

Tokyo RCAI-JSI International Symposium on Immunology. "Exploring and exploiting immunological memory"
Tokyo / *2006*

5th International Congress on Recombinanat Antibodies IBC, "Human monoclonal antibodies from memory B cells."
Zurich, Switzerland / *2006*

Frontiers meeting on Emerging Zoonotic Infections, "Human monoclonal antibodies from memory B cells."
the Wellcome Trust Conference Centre, Hinxton, Cambridge, UK / *2006*

European Congress of Immunology, "Human monoclonal antibodies to treat infectious diseases" and "B cells never forget: monoclonal antibodies from memory B cells."
Paris, France / *2006*

International conference on dendritic cells, "TLR synergy in dendritic cell activation."
Edimburgh, UK / *2006*

Vaccinology Frontiers Meeting, "Human monoclonal antibodies from memory B cells."
The Elvetham, Hartley Wintney, Hampshire, UK
2006

32nd La Jolla Immunology Conference, "The role of TLRs in dendritic cells and B cell activation."
La Jolla, California, USA / *2006*

3rd BIIR Symposium on Human Immunology and Biodefense, "T, B and Dendritic cells."
Houston, USA A. / *2006*

IRB Corso avanzato di formazione sulle neoplasie linfatiche, "Nuovi aspetti dell'immunologia, in particolare nei linfomi."
IRB, Bellinzona / *2006*

Cours d'immunologie approfondie 2006-2007, "T lymphocyte-dendritic cell interactions: intermediates, effector and memory cells."
Institut Pasteur, Paris, France / *2006*

2007

Keystone Symposium, Immunological Intervention in Human Disease, "Exploring and exploiting immunological memory."
Big Sky Resort, Montana, USA / *2007*

Miami Nature Biotechnology Winter Symposium, "Vaccination and immunological memory."
Miami, USA / *2007*

Keystone Symposium: Antibodies as drugs, "Human monoclonal antibodies from memory B cells."
Lake Louise, Canada / *2007*

IELSG Meeting: "T-cell subsets: the physiological basis for a T-cell lymphoma classification."
Lugano, Switzerland / *2007*

Rebavac Workshop "Novel opportunities to develop vaccines to control antibiotic resistant bacteria", "Analytic Vaccinology: will it work against bacteria?"
Siena, Italy / *2007*

Keystone Symposium, Immunologic Memory, Organizers: R. Seder, S. Swain and A. Lanzavecchia. "Innate immunity and the control of CD4 T cell polarization."
Santa Fe, New Mexico / *2007*

DC-Crest workshop: "Dendritic cells and the control of T cell differentiation."
Celerina, Switzerland / *2007*

Lugano Communication Forum,"From basic research to therapeutic antibodies and vaccines."
Lugano, Switzerland / *2007*

WIRM 2007, "Long term T and B cell memory and immune regulation."
Davos, Switzerland / *2007*

3rd Vienna vaccine conference, "Human monoclonal antibodies for passive vaccination and analytic vaccinology."
Baden, Austria / *2007*

5th International Cancer Vaccine Symposium, "Central and effector memory T cells in cancer therapy."
Vienna, Austria / *2007*

Sanquin Spring Seminars, "Human monoclonal antibodies from memory B cells."
the Royal Tropical Insitute in Amsterdam, The Netherlands / *2007*

International Symposium on Molecular Allergology, "On the cellular basis of IgE memory."
Rome, Italy / *2007*

Henry Kunkel Lecture at the Henry Kunkel Society Annual Meeting, "Organizing immunological memory."
The Rockefeller University, NewYork, USA / *2007*

National Institute for Medical Research, "Understanding and making use of human memory B cells."
Mill Hill, London, UK / *2007*

1st Novartis Infectious Disease Meeting, "Human monoclonal antibodies as tools for passive and active vaccination."
Novartis site in Siena, Italy / *2007*

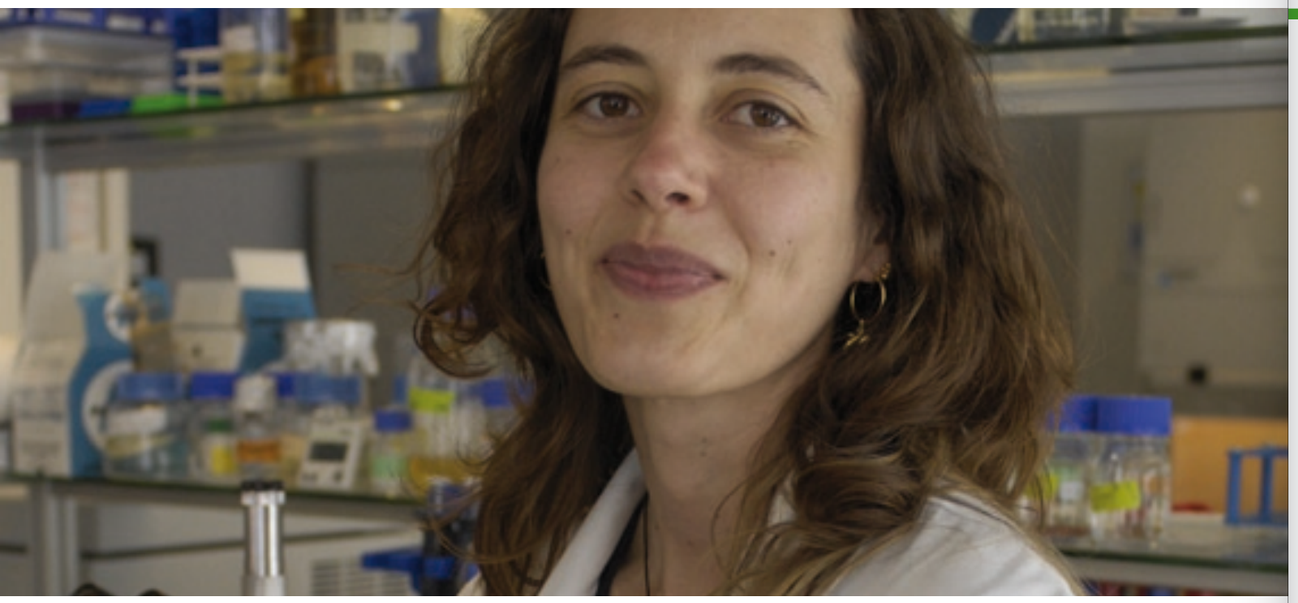
Lettura "Serri", Terzo Congresso Nazionale unificato di Dermatologia e Venereologia, "L'immunità T linfocitaria: dalla protezione alla patologia."
Roma, Italy / *2007*

Riunione IZSV, Individuazione di sinergie medico-veterinarie nell'ambito delle emergenze epidemiche, "Human monoclonal antibodies."
Padova, Italy / *2007*

Keynote lecture, Gaslini advanced course in basic and applied immunology, "Central and effector memory T cells in vaccination and immunotherapy."
Genoa, Italy / *2007*

International Conference on Immunology 2007, "The role of TLRs in dendritic cell and B cell activation."
Shanghai, China / *2007*

microRNA



Silvia Monticelli earned a degree in Biology at the University of Milan where she specialized in Molecular Biology. From July 2000 to January 2007 she was a post-doc in Anjana Rao's laboratory at the Center for Blood Research, Harvard Medical School in Boston (USA), and in February 2007 she joined the IRB as Junior Group Leader.

She did her thesis at the San Raffaele Scientific Institute in Milan with Dr. Donata Vercelli.

Dr. Monticelli has published several papers covering various aspects of the molecular mechanisms underlying the immunopathology of allergy and asthma. Recently she focused her research efforts on the role of microRNAs, a relatively new class of regulatory molecules, in the development and function of cells of the immune system.

Molecular Immunology Lab

During the time spent in Anjana Rao's lab in Boston, I studied the expression, regulation and functions of microRNAs (miRNAs) in cells of the murine immune system.

MiRNAs are small RNA molecules that negatively regulate gene expression; they are derived from short (60-70 nt) hairpin RNAs (pre-miRNAs) that are cleaved from longer primary transcripts (pri-miRNAs). Pri-miRNAs are cleaved into pre-miRNAs by the RNAse Drosha, and exported from the nucleus. In the cytoplasm, a ~22 nt double-stranded (ds)RNA is generated by Dicer. The processed duplex is unwound, and one strand is incorporated into the RNA-induced silencing complex (RISC) machinery. The complex binds to the 3'untranslated region (UTR) of the target mRNAs, inducing a block in translation and/or target mRNA degradation, and therefore reducing the amount of protein being produced.

Biochemical and computational approaches have been used to identify hundreds of miRNAs from various organisms, nevertheless, the role played by miRNAs in the molecular circuitry that controls differentiation of cells of the immune system is still largely unexplored. Furthermore, altered microRNA levels have been discovered in several types of cancer, including leukemia and lymphoma, and there are now many examples of miRNAs functioning as oncogenes or tumor suppressor genes.

At IRB, I am planning to study the mechanisms of miRNA transcriptional and post-transcriptional regulation, as well as the role played by miRNAs in the development and function of cells of the immune system. Specifically, I will investigate the role played by specific miRNAs in mast cells differentiation and function.

Mast cells are specialized cells of the immune system that reside in most tissues, particularly in tissues like the skin that are more exposed to the environment. They act as sentinel cells at sites of antigen entry, and express many inflammatory mediators that affect both innate and adaptive cellular functions of the immune system. Finally, mast cells contribute to pathologic allergic inflammation, but also serve an important protective role in bacterial and parasite infections. Our preliminary results indicate that miRNAs contribute to regulate cell proliferation, and therefore might have a potential role in mastocytosis, which is a tumorigenic transformation of mast cell.

Laboratory

Group Leader: Silvia Monticelli, *PhD, 2007.*

Reference:

• Monticelli S*, Ansel KM, Xiao C, Socci ND, Krichevsky AM, Thai T, Rajewsky N, Marks DS, Sander C, Rajewsky K, Rao A, Kosik KS. *"MicroRNA profiling of the murine hematopoietic system"*. Genome Biology 2005; 6: R71.

**Corresponding author.*

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Multiplying Discovery

Students trained at the IRB continue their scientific careers in institutes and universities around the world.




Education. PhD Programme and PhD students.

The IRB provides high level scientific education for graduate students. The PhD programme, in collaboration with Swiss and foreign universities, includes experimental work carried out at IRB under the direct supervision of a group leader as well as seminars, lessons, and an annual PhD student retreat. Starting from 2004 the Institute organizes an International PhD lecture course that includes lectures and journal clubs. In addition, the Istitute participates to an international PhD programme coordinated by the Vita Salute San Raffaele University of Milan, Italy. The IRB also provides education for undergraduate students (short stages and experimental diploma thesis).

Elena Palmesino

"CXCR4 associated proteins and their role in cell specific receptor function".

Elena Palmesino and Marcus Thelen.

Understanding chemokine receptor-mediated signaling in different cellular environments is the main focus of the project. Ample evidence from our laboratory and by others indicate that coupling of a given G-protein coupled receptor to downstream signaling cascades must be regulated in close proximity to the receptor and may vary between cell types. As a model we investigate the signaling properties of the chemokine receptor CXCR4 which regulates trafficking of leukocytes and tissue cells and is involved in tumor metastasis. The receptor also mediates cell survival and is important in organogenesis. However, CXCR4-stimulated intracellular signaling depends on the cell system. To characterize receptor-associated proteins, that determine the fate of CXCR4-medited cell activation, we developed a solubilization protocol that does not affect the structural integrity of CXCR4, and in which solubilized CXCR4 retains is ability to bind CXCL12. Current investigations should lead to the identification of receptor associated proteins in different cellular systems. Analysis of the expression patterns of the proteins will provide clues on their function in the regulation of CXCR4 activity.

PhD Granted by University of Bern, Switzerland.
Tutor: Professor Andrew Ziemiecki

Simona Infantino

"RDC1, an orphan receptor with similarities to chemokine receptors".

Simona Infantino and Marcus Thelen.

The orphan receptor RDC1 may function as a chemokine receptor. We have characterized its expression pattern in leukocytes, in particular on B cells. So far, we have tested most of the known human chemokines as potential ligands, but could not identify a specific agonist. Receptor internalization on primary and transfected cells was measured as agonist induced response. Recent results obtained in collaboration with our partners in Paris suggest that CXCL12 binds RDC1 and induces its internalization in T cells. However, results form our laboratory indicate that primary B cells do not respond to CXCL12, but to a yet unknown agonist. We assume that the physiological relevant ligand could be an unknown molecule. To this end supernatants derived from different cell culture systems, mimicking the environment where RDC1 positive cells reside, are tested for potential activity on RDC1 and will be fractionated using standard biochemical techniques. Once a ligand has been identified we will study its expression patter and physiological significance.

PhD Granted by University of Bern, Switzerland.
Tutor: Professor Andrew Ziemiecki

Laura Rivino

"Dissecting the human CD4+ T cell memory pool"

Laura Rivino, Antonio Lanzavecchia & Jens Geginat.

Two types of regulatory T cells have been described: "natural" $CD25^+Foxp3^+$ Tregs and adaptive IL-10-secreting "Tr1" cells with unknown phenotype. Induction and maintenance of CCR6 on TCR-activated human T cells required TGF-b and absence of polarising cytokines. In human blood CCR6 was expressed on myeloid DC, on most natural Tregs and on a fraction of antigen-experienced $CD4^+CD25^-Foxp3^-$ T cells. $CD25^-CCR6^+$ T cells possessed high IL-2 and IL-10 producing capacities, but produced IL-10 only following weak TCR stimulation. They were auto-reactive and proliferated with *ex vivo* isolated autologous DC upon IL-10 neutralization in a MHC class II-restricted manner. Moreover, only $CCR6^+$ T cells proliferated with the self-antigen MelanA in healthy donors, while MelanA-reactive cells were predominantly $CCR6^-$ in Viltiligo autoimmune patients. However, $CCR6^+$ T cells responded also to various recall antigens and we isolated several weakly auto-reactive T cell clones that secreted IL-10 upon suboptimal stimulation, but that produced IL-2 and proliferated vigorously upon strong stimulation with tetanus toxoid. We propose that $CCR6^+$ T cells contain context-dependent "Tr1/memory" cells that could behave Tr1-like upon recognition of cross-reactive antigens under steady-state conditions, but act as conventional memory cells in recall responses.

PhD Granted by University of Fribourg, Switzerland.
Tutor: Professor Sandro Rusconi

Roxane Tussiwand

"Flt3 in dentritic cell development"

Roxanne Tussiwand and Markus Manz.

In vivo steady-state type I natural IFN-producing and dendritic cell (DC) development is largely dependent on Flt3 signaling. Natural IFN-producing and DC progenitors and their respective downstream cell populations express the flt3 receptor, and Flt3 ligand (Flt3L)_/_ mice have reduced while Flt3L-injected mice develop markedly increased numbers of both cell types. We could show that SU11657, a small multitargeted receptor tyrosine kinase inhibitor with Flt3 affinity, suppressed *in vitro* natural IFN-producing and DC development in Flt3L-supplemented mouse whole bone marrow cell cultures in a dose dependant manner, while DC development in GM-CSF-supplemented cultures was not affected. *In vivo* SU11657 application led to a significant decrease of both natural IFN-producing and DCs, comparable to the reduction observed in Flt3L_/_ mice. Conversely, Flt3L plasma levels increased massively in inhibitor-treated animals, likely via a regulatory feedback loop, without being able to compensate for pharmacological Flt3 inhibition. No obvious toxicity was observed, and hemopoietic progenitor cell and stem cell function remained intact as assessed by myeloid colony-forming unit activity and *in vivo* bone marrow repopulation assays. Furthermore, upon treatment discontinuation, IFN-producing and DCs recovered to normal levels, proving that treatment effects were transient. DC and IFN-producing cells play an important role in regulation of immune responses and we were able to show that *in vivo* administration of SU11657 could prevent development of EAE in a mouse model system. Collectivly, these findings might lead to new pharmacological strategies in prevention and treatment of autoimmune diseases and complications of organ or blood cell transplantation.
International PhD Program in Basic and Applied Immunology; Università Vita e Salute San Raffaele, Milano

PhD Granted by Università Vita e Salute San Raffaele, Milano, Italy.
Tutor: Professor Ruggero Pardi

Greta Guarda

"Generation and function of mouse central memory and effector memory T cells"

Greta Guarda, Antonio Lanzavecchia and Federica Sallusto.

Based on functional and homing properties, two subsets of memory T lymphocytes have been defined both in humans and in mice. Central memory T cells (TCM cells) express the lymph node homing receptors CD62L and CCR7, have poor effector function and proliferate efficiently upon antigenic stimulation. Effector memory T cells (TEM cells) do not express CCR7, are mostly CD62L negative and therefore are excluded from lymph nodes, but are able to migrate to sites of inflammation where they exert immediate effector function by producing inflammatory cytokines and cytotoxic mediators.

In the present work we have addressed two questions that emerged since the definition of TCM and TEM cells. Firstly, what are the priming conditions for generation of TCM and TEM and, secondly, what is the migratory capacity of TCM and TEM cells in inflammatory conditions. By using naive TCR-transgenic OT-I CD8+ T cells and OT-II CD4+ T cells and ovalbumin pulsed-mature dendritic cells (DCs) we set up an *in vitro* system in which the strength of T cell stimulation is controlled by varying the ratio of T cells and DCs and the duration of DC-T cell interaction. Using this system we found that precursors of TCM and TEM cells are generated at different strength of stimulation and that T cells capable of persisting *in vivo* in the absence of antigen and of mounting recall responses is optimally induced by intermediate stimulatory strength. In addition, we found that lymph nodes draining sites of mature DC or adjuvant inoculation recruit CD8+ CD62Lγ CCR7γeffector and TEM cells. CD8+ T cell recruitment in reactive lymph nodes requires CXCR3 expression on T cells and occurs through high endothelial venules (HEVs) in concert with HEV luminal expression of the CXCR3 ligand CXCL9. In reactive lymph nodes, recruited T cells establish stable interactions with and kill antigen-bearing DCs, limiting the ability of these DCs to activate CD4+ and CD8+ T cells. Taken togther these data define conditions for the generation of TCM and TEM cells and define an inflammatory pathway of effector T cell migration in lymph nodes. The inducible recruitment of blood-borne effector and TEM CD8+ cells to lymph nodes may represent a mechanism for terminating primary and limiting secondary immune responses.

PhD Granted by University of Lausanne, Switzerland
Tutor: Professor Hans Acha-Orbea

Claudia Ruprecht

"On the role of regulatory T cells and microbial products in the control of T and B cell immune responses"

Claudia Ruprecht and Antonio Lanzavecchia.

Self-nonself discrimination is the basic property of the immune system that allows rejection of pathogens without attacking self-specific structures. Discrimination of self and nonself is based on both structural features of the antigen as well as on the context, in which the antigen is encountered. While specific recognition of nonself-antigens in presence of microbial products induces potent immune responses, several suppressing mechanisms exist that limit immune reactions to specifically recognized antigens in a context devoid of microbial agents.

A prominent example of suppressing mechanisms is regulation of T cell responses by regulatory T cells (Tregs). Treg-mediated suppression is induced upon T cell receptor (TCR) stimulation of Tregs and therefore dependent on Treg specificity. We found that TCRs derived from mouse regulatory and conventional T cells cover a similar spectrum of affinity towards self-antigens, which implies that Tregs express a similar TCR repertoire as conventional T cells. This result suggests that Treg-mediated suppression is not induced by recognition of self-antigen but rather regulated by recognition of the immunological context.

Characterization of Treg function in autoimmune diseases is hampered by the fact that Tregs in an inflamed tissue cannot be discriminated from infiltrating activated conventional T cells. We report that at the site of autoimmune reactions Tregs can be distinguished from activated T cells by the expression of CD27. Using this novel Treg marker we show that the suppressive activity of Tregs isolated from inflamed tissues is not limited *in vitro*, which precludes a Treg-intrinsic defect. However we have observed that cytokines as IL-7 and IL-15, which are present in the autoimmune inflammatory milieu, potently block Treg-mediated suppression *in vitro*. These results suggest that *in vivo* IL-7 and IL-15 may interfere with Treg function at the sites of ongoing autoimmune reactions.

Recognition of a context containing signs of microbial invasion leads to the counterbalancing of suppressing mechanisms and to the induction of potent immune responses. Such a context is characterized by the presence of pathogen-associated molecular patterns (PAMPs) that are recognized by Toll-like receptors (TLRs) expressed on a variety of cell types. We show that TLR triggering is critically required for the induction of productive T-dependent human naïve B cell responses. B cell receptor (BCR) triggering and T cell help induced initial B cell proliferation but were not sufficient to sustain prolonged survival and accumulation of B cells. Extensive proliferation, isotypic switch and differentiation to Ig-secreting cells were promoted by microbial agents acting on TLRs expressed by naïve B cells upon BCR stimulation. This finding demonstrates that humoral immune responses (as cellular immune responses) are critically dependent on context discrimination via detection of PAMPs.

PhD Granted by University of Basel, Switzerland.
Tutor: ***Professor Antonius Rolink***

Silvia Olivari

"EDEM1, EDEM2 and EDEM3 as ER-Associated Degradation Regulators"

Silvia Olivari and Maurizio Molinari

Protein folding is error prone and, under physiological conditions, only a fraction of newly synthesized proteins folds correctly. Misfolded proteins are potentially dangerous for the cell homeostasis. Thus, they must be recognized and destroyed. Inefficient removal of the misfolded proteins can generate several "gain of function" diseases, such as the amyloidosis, that affect an increasing number of patients. On the other hand, removal of potentially functional proteins carrying structural defects causes several "loss-of-function" diseases such as cystic fibrosis. Consequently, understanding the mechanisms of the ER associated degradation, ERAD, is of great interest and has been the aim of my studies.

EDEM1 was described as a type-II ER transmembrane protein belonging to the 47 glycosyl hydrolase family (GH47). Its involvement in ERAD was already assessed such as its up-regulation during the unfolded protein response, UPR. We used the amino acid sequence of the EDEM1 GH47 domain as probe and identified, in the National Center for Biotechnology Information (NCBI) created data base, two unknown proteins. Alignment between EDEM1 and those two novel proteins, that we named EDEM2 and EDEM3, shows a ≈44% identity restricted to the GH47 domain.

We demonstrate that both EDEM2 and EDEM3 are up-regulated during UPR via the IRE1/Xbp1 pathway. Our results show that EDEM2 is a luminal protein of the ER and that its up-regulation accelerates the degradation of misfolded proteins. We also demonstrate that EDEM1 is a luminal protein rather than a type-II ER transmembrane protein as previously described.

EDEM1 was described as a putative low mannose binding lectin without detectable glycanase activity. Lack of enzymatic activity was attributed to the lack of a cysteine in the putative active site that is conserved in many, but not all, active mannosidases.

We describe EDEM1 as a chaperone that inhibits covalent aggregation of misfolded polypeptides and as a putative active mannosidase. We demonstrate that the overexpression of EDEM1 accelerates the removal of one or more mannose residues specifically from the glycan belonging to folding defective proteins (as no interaction between EDEM1 and a folding competent substrate has been seen). Notably all the catalytic amino acids of the ER 1,2 alpha mannosidase I are spatially conserved in the EDEM1 structure. Acceleration of substrates de-mannosylation but not the chaperone activity of EDEM1 were abolished by mutations of a catalytic residue conserved in the ER 1,2 alpha mannosidase I. Similar results were published for the EDEM3 protein by the group of Nagata.

Our finding that EDEM1 also accelerates substrate de-mannosylation in a cell line (B3F7) where the glycan added to the nascent polypeptides lacks mannoses on both branches "B" and "C" demonstrates its ability in removing the mannose on branch "A". This data supports a model in which extensive de-mannosylation is required for ERAD.

PhD Granted by University of Fribourg, Switzerland.
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"Cell Biology of dendritic cells."
Dept. Cell Biology, Ludwig Institute for cancer Research Yale, University School of Medicine, New Haven, USA
01.12.2006

Rolf Zinkernage
"Against dogma and against feelings."
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Universitätsspital Basel, Dept. Forschung Experimentelle, Hämatologie, Basel, Switzerland 20.04.2007

Ton Schumacher
The Netherlands Cancer Institute, Dept. of Immunology, Amsterdam, The Netherlands 24.05.2007

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Immunopathogenesis of Liver Infections Unit, DIBIT, San Raffaele Scientific Institute, Milan, Italy / *11.01.2005*

Kurt Ballmer-Hofer – "From Receptor/Ligand Interaction to Modulation of Cell Junctions."
Molecular Cell Biology, Paul Scherrer Institut, Villigen-PSI, Switzerland / *18.01.2005*

Philippe Moingeon – "Challenges and opportunities in the development of recombinant allergy vaccines."
Research and Development Stallergenes, Antony, France / *24.01.05*

Marco Bianchi – "HMGB1 is the signal for traumatic cell death."
Molecular Biology, San Raffaele University, Milan, Italy / *31.01.2005*

Klaus Uberla – "Lentiviral vectors for gene therapy and vaccination."
Department of Molecular and Medical Virology, Ruhr-University Bochum, Germany / *08.03.2005*

Albrecht Wendel – "Membrane-bound TNF mediates melphalan hepatotoxicity via activation of both TNF receptors"
Biochemical Pharmacology, University of Konstanz, Konstanz, Germany / *11.03.2005*

Carlomaurizio Montecucco – "Rheumatoid arthritis: clinical perspectives."
Cattedra e Struttura Complessa di Reumatologia, Scuola di Specializzazione in Reumatologia, Università di Pavia, Policlinico S. Matteo, Pavia, Italy / *16.03.2005*

Gerardo Z. Lederkremer – "Distinct sugar chain processing and sorting in ER quality control."
Department of Cell Research and Immunology, Faculty of Life Sciences, Tel Aviv University, Israel / *04.04.2005*

Werner Muller-Esterl – "The nuts and bolts of the nitric oxide (NO) signaling cascade." Institute for Biochemistry, University of Frankfurt Medical School, Frankfurt, Germany / *07.04.2005*

Florian Bihl – "Immune dominant cytotoxic T lymphocyte responses in viral co-infections"
Partners AIDS Research Center, Massachussetts General Hospital, Harvard Medical School, Charlestown, Boston, USA / *07.04.2005*

Bernhard Moser – "Role of chemokines in antigen presentation"
Institute of Cell Biology, University of Bern, Switzerland / *12.04.2005*

Grazia Galli – "Acquiring from the innate: NKT cells help to B cell responses"
Immunology and Virology Unit, Chiron Vaccines Srl, Siena, Italy / 13.04.2005

Fulvio Reggiori – "Membrane trafficking during autophagosome formation."
University of Michigan, Life Sciences Institute, Ann Arbor, USA / *15.04.2005*

Nick Huntington – "Regulation of B and NK cell immune responses by CD45."
Immunology Division, Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia *25.04.2005*

Rhys Allan – "Re-examining the Role of Skin Dendritic Cells in CTL Immunity to HSV-1 infection."
Department of Microbiology and Immunology, University of Melbourne, Australia / *27.04.2005*

Antal Rot – "Chemokine Interceptors: Silent molecules with different functions"
Novartis Institute for Biomedical Research, Vienna, Austria / *11.05.2005*

Tanja Hartmann – "CXCR4 chemokine receptor and b1 signaling cooperate in mediateing adhesion and chemoresistance in small cell lung cancer and chronic lymphocytic leukaemia."
Department of Internal Medicine, University Hospital Freiburg, Germany / *13.05.2005*

Sanjiv Luther – "Lymphoid tissue development and cell migration"
Department of Biochemistry, University of Lausanne, Epalinges, Switzerland / *23.05.2005*

Antonio G. Siccardi –"IgE enhance the immunogenicity of cellular tumor vaccines"DIBIT-HSR, Milan, Italy / *01.06.2005*

Jens V. Stein – "Intracellular control of lymphocyte trafficking."
Theodor Kocher Institute, University of Bern, Switzerland / *02.06.2005*

Lars Hviid – "Malaria Immunology"
Centre for Medical Parasitology, Department of Infectious Diseases, Rigshospitalet, Copenhagen, Denmark / *14.06.2005*

Michael T. Lotze – "Dying Dangerously: Apoptosis, Necrosis and Cancer."
Translational Research, Molecular Medicine Institute, University of Pittsburgh School of Medicine, Pittsburgh, USA / *15.06.2005*

Ronen Alon – "Chemokine activation of leukocyte integrins under shear flow: new findings and more puzzles"
Department of Immunology, Rehovot, Israel *05.07.2005*

Ruth Ruprecht – "Harnessing the Power of Neutralizing Monocolonal Antibody Combinations Against HIV"
Dana-Farber Cancer Institute, Boston, USA *07.07.2005*

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Universitätsklinikum Aachen, Institut für Biochemie, Aachen, Germany / *08.07.2005*

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Ruprecht-Karls Universität Heidelberg, Heidelberg, Germany / *15.07.2005*

Hans-Georg Rammensee – "Peptide selection for vaccination of cancer patients" Interfakultäres Institut für Zellbiologie, Abt. Immunologie, Tübingen, Germany / *18.07.2005*

Linda M. Hendershot – "The unfolded protein response and its role in plasma cell differentiation"
Department of Genetics and Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN, USA / *04.10.2005*

Joost J. Oppenheim – "Non cognate ligands of chemokine receptors: autoantigens, tumorantigens and alarmins"
Laboratory of Molecular Im munoregulation, National Cancer Institute, Frederick, USA *05.10.2005*

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CBR Institute for Biomedical Research, Harvard Medical School, Boston, USA / *17.10.2005*

Jean-Yves Salomone – "Novel applications for Genomic analysis using genechip microarrays"
Affymetrix, High Wycombe, UK / *18.10.2005*

Tim Willinger – "A molecular view of human CD8 T cell memory subsets"
Weatherall Institute of Molecular Medicine, MRC Human Immunology Unit, John Radcliffe Hospital, Oxford, UK / *20.10.2005*

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Tumor Immunology Programme, Division of Cellular Immunology, DKFZ, Heidelberg, Germany / *25.10.2005*

Thomas Simmen – "A novel ER Signaling Pathway to Mitochondria"
Department of Cell Biology, University of Alberta, Edmonton, Canada / *27.10.05*

Colin Watts – "Antigen capture and processing by professional antigen presenting cells"
Wellcome Trust Biocentre, School of Life Sciences University of Dundee, Dundee, UK *10.11.2005*

Caetano Reis e Sousa – "Innate Sensing of Infection by Dendritic Cells"
Immunobiology Laboratory, London Research Institute, Lincoln's Inn Fields Laboratories, London, UK / *24.11.2005*

Assioma SA – "Bioinformatics: tools for the lab"
Lugano, Switzerland / *30.11.2005*

Mauro Delorenzi – "GGI (Gene Expression Grade Index): a Method to grade Breast Cancer with Expression Profiles and a robust Prognostic factor"
NCCR Molecular Oncology Program at ISREC, Lausanne, Switzerland / *19.12.2005*

2006

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Ambion Europe Ltd / *10.01.2006*

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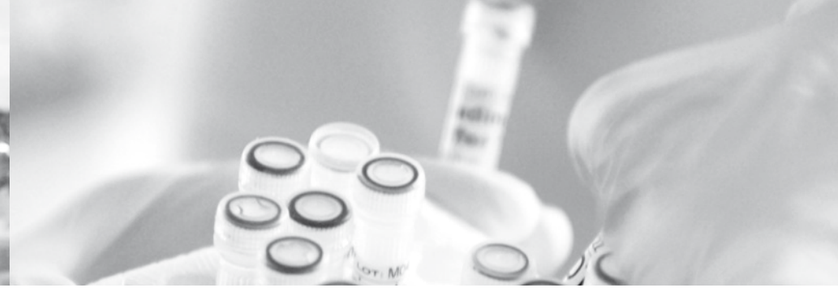
Immunology Department, Weizmann Institute of Science, Rehovot, Israel / *26.01.2006*







Ingmar AFM Heijnen "Flow cytometric disease monitoring in B-cell neoplasms"
Zentrum für Labormedizin, Kantonsspital Aarau, Aarau, Switzerland / *31.01.2006*

Luca Scorrano –"Keeping mitochondria in shape: a matter of life and death"
Venetian Insti-tute of Molecular Medicine, Dulbecco Telethon Institute, Padua, Italy
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Department of Immunology, The Scripps Research Institute, La Jolla, CA, USA / *09.02.2006*

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Division of Infectious Diseases and Hospital Epidemiology Department of Medicine, University Hospital Zurich, Switzerland / *23.02.2006*

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Department of Obstetrics, University Hospital Zurich, Switzerland / *28.02.2006*

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Division of Pathology and Laboratory Medicine, European Institute of Oncology, Milan, Italy
07.03.2006

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Division of Hematology Unit, Dept. of Medical Sciences and IRCAD, A. Avogadro Univ. of Eastern Piedmont, Novara, Italy / *15.03.2006*

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Sir William Dunn School of Pathology, University of Oxford, UK / *29.03.2006*

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Cell Biology & Physiology, University of New Mexico, Albuquerque, Mexico / *05.04.2006*

Applied Biosystems –"Gene expression and Genotyping"
Applera Europe B.V., Rotkreuz Branch, Rotkreuy, Switzerland / 13.04.2006

Marisa Jaconi – "Human embryonic stem cells, cardiac differentiation and therapies of the heart: the future is now?"
Department of Pathology and Immunology Faculty of Medecine, Geneva University, Switzerland / *21.04.2006*

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Onur Boyman, "Antibody-cytokine complexes as tools to modulate immune responses" Div. of Immunology and Allergology, Dept. of Medicine, University Hospital of Lausanne, Switzerland / *06.07.2006*

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INSERM U503, Centre d'Etudes et de Recherches en Virologie et Immunologie, Lyon, France
17.07.2006

Martin Lohse – "Optical recording of receptor activation and signalling"
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SPRI, Serono International SA, Geneva, Switzerland / *24.08.2006*

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Departments of Pathology and Medicine, University of California San Diego, USA / *29.08.2006*

Claudia Lengerke – "Patterning hematopoiesis in embryonic stem cells"
Children's Hospital, Boston, USA / *12.09.2006*

Anna Mondino – "The making and breaking of tumor-specific T cell memory" Biotechnology School, Vita-Salute San Raffaele University, Italy / *10.10.2006*

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CISRO Livestock Industries, Armidale, Australia
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Dept. of Medicine, Faculty of Science, University of Fribourg / *02.07.2007*

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Financial Data 2006

Core funding of the IRB is provided by public and private donors; The Helmut Horten Foundation, The Confederation, The Canton of Ticino and the City of Bellinzona, as well as individual donors.

Balance Sheet as 31 of December 2006

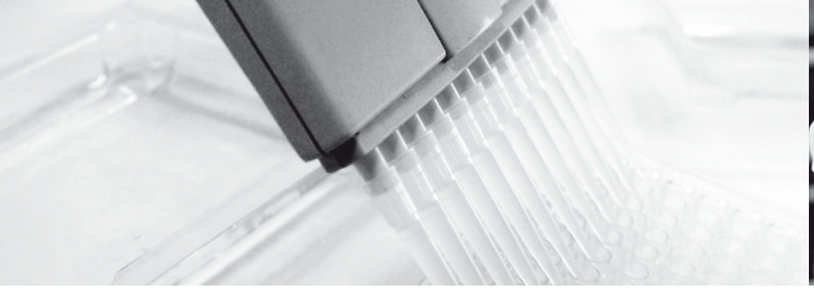
ASSETS		31.12.2006	31.12.2005
1.	*Liquidity*	2,394,252	3,777,159
2.	*Various Receivables*	1,915,242	612,987
3.	*Temporary Receivables*	1,034,999	1,297,686
	Current Assets	**5,344,493**	**5,687,833**
4.	*Buildings*	5,274,944	4,317,440
5.	*Furnishing & Equipment*	3,583,000	4,078,000
	Fixed Assets	**8,857,944**	**8,395,440**
	TOTAL ASSETS	14,202,438	14,083,273

LIABILITIES		31.12.2006	31.12.2005
1.	*Debt for delivery and services*	487,441	465,715
2.	*Accruals*	272,979	575,164
3.	*Funds for Research Projects*	1,199,979	587,274
4.	*Funds for Laboratories*	1,370,249	1,328,398
5.	*Various Funds*	264,996	189,996
	Current Liabilities	**3,595,645**	**3,146,547**
6.	*Long Term Loans*	3,800,000	2,800,000
	Long Term Liabilities	**3,800,000**	**2,800,000**
7.	*Capital Resources*	8,136,726	9,946,355
8.	*Annual Result*	-1,329,932	-1,809,629
	Equity of the Foundation	**6,806,793**	**8,136,726**
	TOTAL LIABILITIES	14,202,438	14,083,273

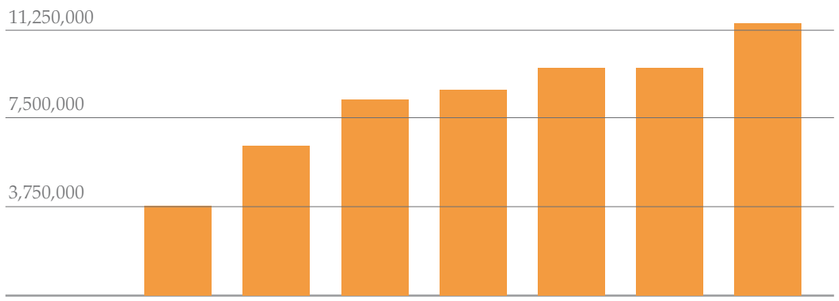
Profit and Loss Account for the year 2005 and 2006 (In Swiss Francs)

COSTS		31.12.2006	31.12.2005
1.	*Personnel Costs*	4,903,221	4,613,152
2.	*Consumables*	1,677,051	1,559,230
3.	*Maintenance of Buildings and Equipment*	359,778	283,142
4.	*Investments*	677,524	145,023
5.	*Amortizations*	674,135	678,451
6.	*Rent and related Costs*	883,886	877,692
7.	*Administrative Costs and Various*	974,792	1,205,714
8.	*Travel, Congresses and Guests*	306,606	161,071
9.	*Various costs for Research*	851,908	238,742
	Total Costs	**11,308,902**	**9,762,217**
10.	*Contributions from the Confederation*	1,105,000	690,000
12.	*Contributions from the City of Bellinzona*	500,000	500,000
13.	*Contributions from the Helmut Horten Fnd.*	1,500,000	1,500,000
14.	*Other Contributions*	974,706	964,428
15.	*Research Projects*	4,851,391	3,734,124
16.	*Other Revenue*	1,047,872	564,035
	Totale revenue	**9,978,969**	**7,952,588**
	ANNUAL RESULT	1,329,932	1,809,629

The annual result is covered by the capital resurces of the Foundation financed mainly by the Canton Ticino.

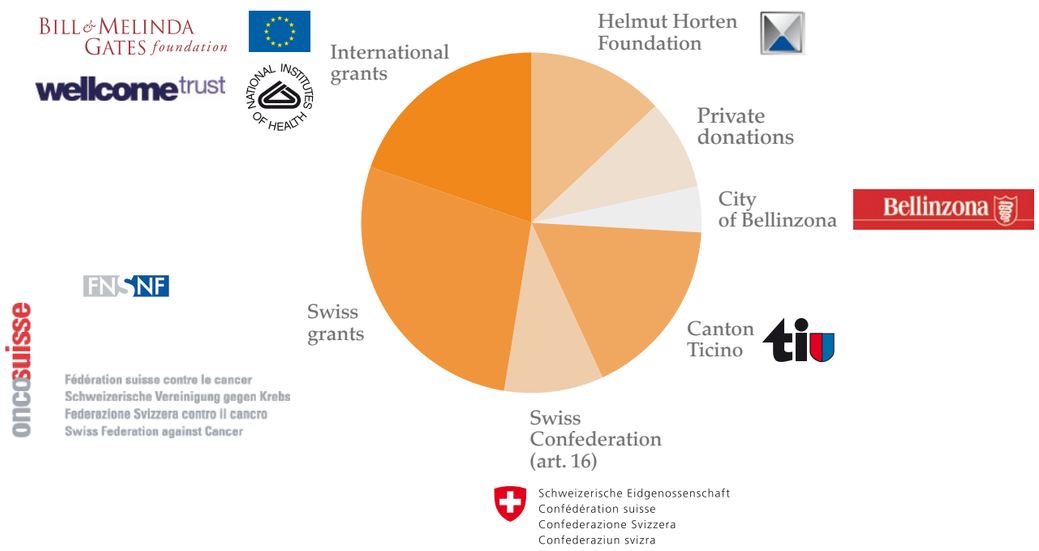


Evolution of Costs 2000-2006



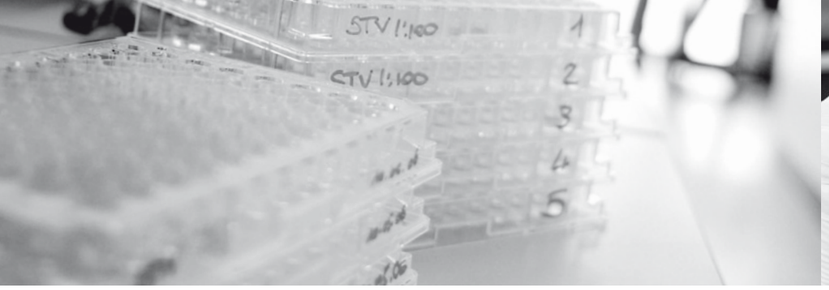
COSTS	2000	2001	2002	2003	2004	2005	2006
Personnel	1,825,910	3,456,425	4,498,136	4,749,499	5,008,522	4,613,152	4,903,221
General	1,991,623	2,763,354	3,883,763	3,958,932	4,789,958	5,149,065	6,405,681
TOT. COSTS	3,817,533	6,219,779	8,381,899	8,708,431	9,798,480	9,762,217	11,308,902

Funding by Source



Contributing Discovery

Knowledge created at the IRB impacts understanding of immunology through publications, seminars and the constant movement of IRB students and scientists.



Publications 2000-2007

This list includes the IRB publications from 2005 to 2007.

The average Impact Factor is 11.9.

2005

105. Dendritic cell-derived IL-2 production is regulated by IL-15 in humans and in mice.
Feau S., Facchinetti V., Granucci F., Citterio S., Jarrossay D., Seresini S., Protti M. P., Lanzavecchia A., and Ricciardi-Castagnoli P. / *Blood 2005; 105:697-702*
IF: 10.1

106. A rich chemokine environment strongly enhances leukocyte migration and activities
Paoletti S., Petkovic V., Sebastiani S., Danelon M. G., Uguccioni M., and Gerber B. O. / *Blood 2005; 105:3405-3412*
IF: 10.1

107. A novel stress-induced EDEM variant regulating endoplasmic reticulum-associated glycoprotein degradation
Olivari S., Galli C., Alanen H., Ruddock L., and Molinari M. / *J Biol Chem 2005; 280:2424-2428*
IF: 5.9

108. Sliding doors in the immune response
Sallusto F. and Reiner S. L. / *Nat Immunol 2005; 6:10-12*
IF: 27.0

109. Arginine methyltransferase CARM1 is a promoter-specific regulator of NF-kappaB-dependent gene expression
Covic M., Hassa P. O., Saccani S., Buerki C., Meier N. I., Lombardi C., Imhof R., Bedford M. T., Natoli G., and Hottiger M. O. / *EMBO J 2005; 24:85-96*
IF: 10.1

110. Evasion of antibody neutralization in emerging severe acute respiratory syndrome coronaviruses.
Yang Z.Y., Werner H. C., Kong W. P., Leung K., Traggiai E., Lanzavecchia A., and Nabel G. J.
Proc Natl Acad Sci U S A 2005; 102:797-801
IF: 10.2

111. CCL22-induced responses are powerfully enhanced by synergy inducing chemokines via CCR4: evidence for the involvement of first beta-strand of chemokine.
Sebastiani S., Danelon G., Gerber B., and Uguccioni M. / *Eur J Immunol 2005; 35:746-756*
IF: 4.9

112. Degradation of trafficking-defective long QT syndrome type II mutant channels by the ubiquitin-proteasome pathway.
Gong Q., Keeney D. R., Molinari M., and Zhou Z. / *J Biol Chem 2005; 280:19419-19425*
IF: 5.9

113. beta-site specific intrabodies to decrease and prevent generation of Alzheimer's Abeta peptide.
Paganetti P., Calanca V., Galli C., Stefani M., and Molinari M. / *J Cell Biol 2005; 168:863-868*
IF: 11.0

114. Immune subversion by Helicobacter pylori.
Baldari C. T., Lanzavecchia A., and Telford J. L.
Trends Immunol 2005; 26:199-207
IF: 10.2

115. Dendritic cells as a tool for the predictive identification of skin sensitisation hazard.
Casati S., Aeby P., Basketter D. A., Cavani A., Gennari A., Gerberick G. F., Griem P., Hartung T., Kimber I., Lepoittevin J. P., Meade B. J., Pallardy M., Rougier N., Rousset F., Rubinstenn G., Sallusto F., Verheyen G. R., and Zuang V.
Altern Lab Anim 2005; 33:47-62
IF: 0.7

116. T cell costimulation by chemokine receptors.
Molon B., Gri G., Bettella M., Gomez-Mouton C., Lanzavecchia A., Martinez A., Manes S., and Viola A. / *Nat Immunol 2005; 6:465-471*
IF: 27.0

117. Systematic microanatomical analysis of CXCL13 and CCL21 in situ production and progressive lymphoid organization in rheumatoid synovitis.
Manzo A., Paoletti S., Carulli M., Blades M. C., Barone F., Yanni G., Fitzgerald O., Bresnihan B., Caporali R., Montecucco C., Uguccioni M., and Pitzalis C. / *Eur J Immunol 2005; 35:1347-1359*
IF: 4.9

118. Toll-like receptor-dependent activation of several human blood cell types by protamine-condensed mRNA.
Scheel B., Teufel R., Probst J., Carralot J. P., Geginat J., Radsak M., Jarrossay D., Wagner H., Jung G., Rammensee H. G., Hoerr I., and Pascolo S.
Eur J Immunol 2005; 35:1557-1566
IF: 4.9

119. Interactions of NF-kappaB with chromatin: the art of being at the right place at the right time.
Natoli G., Saccani S., Bosisio D., and Marazzi I.
Nat Immunol 2005; 6:439-445
IF: 27.0

120. Understanding the generation and function of memory T cell subsets
Lanzavecchia A. and Sallusto F. / *Curr Opin Immunol 2005; 17:326-332*
IF: 9.1

121. The glycan code of the endoplasmic reticulum: asparagine-linked carbohydrates as protein maturation and quality-control tags.
Hebert D. N., Garman S. C., and Molinari M.
Trends Cell Biol 2005; 15:364-370
IF: 11.8

122. Coexpression of CD25 and CD27 identifies FoxP3+ regulatory T cells in inflamed synovia.
Ruprecht C. R., Gattorno M., Ferlito F., Gregorio A., Martini A., Lanzavecchia A., and Sallusto F.
J Exp Med 2005; 201:1793-1803
IF: 14.0

123. The use of calnexin and calreticulin by cellular and viral glycoproteins.
Pieren M., Galli C., Denzel A., and Molinari M.
J Biol Chem 2005; 280:28265-28271
IF: 5.9

124. Human Adaptive Immune System Rag2-/-{gamma}c-/- Mice
Chicha L., Tussiwand R., Traggiai E., Mazzucchelli L., Bronz L., Piffaretti J. C., Lanzavecchia A., and Manz M. G. / *Ann N Y Acad Sci 2005; 1044:236-243*
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125. Expression of lymphocyte activation gene 3 (LAG-3) on B cells is induced by T cells.
Kisielow M., Kisielow J., Capoferri-Sollami G., and Karjalainen K. / *Eur J Immunol 2005; 35:2081-2088*
IF: 4.9

126. Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells.
Napolitani G., Rinaldi A., Bertoni F., Sallusto F., and Lanzavecchia A. / *Nat Immunol 2005; 6:769-776*
IF: 27.0

127. CD molecules 2005: human cell differentiation molecules.
Zola H., Swart B., Nicholson I., Aasted B., Bensussan A., Boumsell L., Buckley C., Clark G., Drbal K., Engel P., Hart D., Horejsi V., Isacke C., Macardle P., Malavasi F., Mason D., Olive D., Saalmueller A., Schlossman S. F., Schwartz-Albiez R., Simmons P., Tedder T. F., Uguccioni M., and Warren H. / *Blood 2005; 106:3123-3126*
IF: 10.1

128. The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes
Balabanian K., Lagane B., Infantino S., Chow K. Y., Harriague J., Moepps B., renzana-Seisdedos F., Thelen M., and Bachelerie F. / *J Biol Chem 2005; 280:35760-35766*
IF: 5.9

129. Inhibition of natural type I IFN-producing and dendritic cell development by a small molecule receptor tyrosine kinase inhibitor with Flt3 affinity.
Tussiwand R., Onai N., Mazzucchelli L., and Manz M. G. / *J Immunol 2005; 175:3674-3680*
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130. A c-Rel subdomain responsible for enhanced DNA-binding affinity and selective gene activation.
Sanjabi S., Williams K. J., Saccani S., Zhou L., Hoffmann A., Ghosh G., Gerondakis S., Natoli G., and Smale S. T. / *Genes Dev 2005; 19:2138-2151*
IF: 15.6

131. P-rex1 regulates neutrophil function
Welch H. C., Condliffe A. M., Milne L. J., Ferguson G. J., Hill K., Webb L. M., Okkenhaug K., Coadwell W. J., Andrews S. R., Thelen M., Jones G. E., Hawkins P. T., and Stephens L. R. / *Curr Biol 2005; 15:1867-1873*
IF: 11.7

132. ABCB1 transporter discriminates human resting naive B cells from cycling transitional and memory B cells.
Wirths S. and Lanzavecchia A. / *Eur J Immunol 2005; 35:3433-3441*
IF: 4.9

133. Humanized mice: are we there yet?
Macchiarini F., Manz M. G., Palucka A. K., and Shultz L. D. / *J Exp Med 2005; 202:1307-1311*
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134. Analyzing folding and degradation of metabolically labelled polypeptides by conventional and diagonal SDS-polyacrylamide gel electrophoresis
Soldà T., Olivari S., and Molinari M. / *Biological Procedures Online 2005; 7:136-143*
IF: N/A

135. Persistent glycoprotein misfolding activates the glucosidase II/UGT1-driven calnexin cycle to delay aggregation and loss of folding competence.
Molinari M., Galli C., Vanoni O., Arnold S. M., and Kaufman R. J. / *Mol Cell 2005; 20:503-512*
IF: 15.0

136. Involvement of Prep1 in the alphabeta T-cell receptor T-lymphocytic potential of hematopoietic precursors.
Penkov D., Di Rosa P., Fernandez Diaz L., Basso V., Ferretti E., Grassi F., Mondino A., and Blasi F.
Mol Cell Biol 2005; 25:10768-10781
IF: 7.1

137. Identification of T cell-restricted genes, and signatures for different T cell responses, using a comprehensive collection of microarray datasets.
Chtanova T., Newton R., Liu S. M., Weininger L., Young T. R., Silva D. G., Bertoni F., Rinaldi A., Chappaz S., Sallusto F., Rolph M. S., and Ma W ckay C. R. / *J Immunol 2005; 175:7837-7847*
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138. The secretory capacity of a cell depends on the efficiency of endoplasmic reticulum-associated degradation.
Molinari M. and Sitia R. / *Curr Top Microbiol Immunol 2005; 300:1-15*
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2006

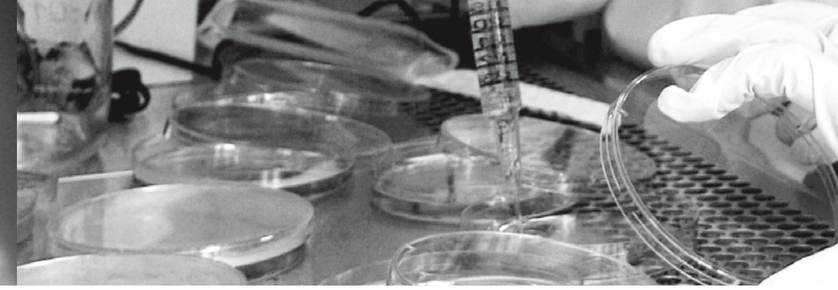
139. Consequences of ERp57 deletion on oxidative folding of obligate and facultative clients of the calnexin cycle.
Solda T., Garbi N., Hammerling G. J., and Molinari M. / *J Biol Chem 2006; 281:6219-6226*
IF: 5.9

140. Activation of the Flt3 signal transduction cascade rescues and enhances type I interferon-producing and dendritic cell development.
Onai N., Obata-Onai A., Tussiwand R., Lanzavecchia A., and Manz M. G. / *J Exp Med 2006; 203:227-238*
IF: 14.0

141. Characterization of severe acute respiratory syndrome coronavirus membrane protein.
Voss D., Kern A., Traggiai E., Eickmann M., Stadler K., Lanzavecchia A., and Becker S.
FEBS Lett 2006; 580:968-973
IF: 3.4

142. Plastic downregulation of the transcriptional repressor BCL6 during maturation of human dendritic cells.
Pantano S., Jarrossay D., Saccani S., Bosisio D., and Natoli G. / *Exp Cell Res 2006; 312:1312-1322*
IF: 4.1





143. Expression and regulation of the orphan receptor RDC1 and its putative ligand in human dendritic and B cells.
Infantino S., Moepps B., and Thelen M.
J Immunol 2006; 176:2197-2207
IF: 6.4

144. A hyper-dynamic equilibrium between promoter-bound and nucleoplasmic dimers controls NF-kappaB-dependent gene activity
Bosisio D., Marazzi I., Agresti A., Shimizu N., Bianchi M.E., Natoli G. / *EMBO J 2006; 25:798-810*
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145. Regulation of peripheral T cell activation by calreticulin.
Porcellini S., Traggiai E., Schenk U., Ferrera D., Matteoli M., Lanzavecchia A., Michalak M., and Grassi F. / *J Exp Med 2006; 203:461-471*
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146. Notch1-dependent lymphomagenesis is assisted by but does not essentially require pre-TCR signaling.
Campese A. F., Garbe A. I., Zhang F., Grassi F., Screpanti I., and von Boehmer H. / *Blood 2006; 108:305-310*
IF: 10.1

147. *Toll-like receptor stimulation as a third signal required for activation of human naive B cells.*
Ruprecht C. R. and Lanzavecchia A. / *Eur J Immunol 2006; 36:810-816*
IF: 4.9

148. Differences in CXCR4-mediated signaling in B cells.
Palmesino E., Moepps B., Gierschik P., and Thelen M. / *Immunobiology 2006; 211:377-389*
IF: 1.8

149. A cyanobacterial LPS antagonist prevents endotoxin shock and blocks sustained TLR4 stimulation required for cytokine expression.
Macagno A., Molteni M., Rinaldi A., Bertoni F., Lanzavecchia A., Rossetti C., and Sallusto F.
J Exp Med 2006; 203:1481-1492
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150. Follicular B helper T cell activity is confined to CXCR5(hi)ICOS(hi) CD4 T cells and is independent of CD57 expression.
Rasheed A. U., Rahn H. P., Sallusto F., Lipp M., and Muller G. / *Eur J Immunol 2006; 36:1892-1903*
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151. Cyclophilin, TRIM5, and innate immunity to HIV-1.
Sokolskaja E. and Luban J. / *Curr Opin Microbiol 2006, 9:404-408*
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Lanzavecchia A., Bernasconi N., Traggiai E., Ruprecht C.R., Corti D., and Sallusto F. / *Immunol Rev 2006; 211:303-309*
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153. A new role for CCR5 in innate immunity--binding to bacterial heat shock protein 70.
Mackay C. R. and Sallusto F. / *Eur J Immunol 2006; 36:2293-2295*
IF: 4.9

154. N-linked glycan recognition and processing: the molecular basis of endoplasmic reticulum quality control.
Moremen K. W. and Molinari M. / *Curr Opin Struct Biol 2006; 16:592-599*
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155. EDEM1 regulates ER-associated degradation by accelerating de-mannosylation of folding-defective polypeptides and by inhibiting their covalent aggregation.
Olivari S., Cali T., Salo K. E., Paganetti P., Ruddock L. W., and Molinari M. / *Biochem Biophys Res Commun 2006; 349:1278*-1284
IF: 3.0

156. Microbiology: death of a chaperone.
Montecucco C. and Molinari M. / *Nature 2006; 443:511-512*
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157. Disseminated and sustained HIV infection in CD34+ cord blood cell-transplanted Rag2-/-gamma c-/- mice.
Baenziger S., Tussiwand R., Schlaepfer E., Mazzucchelli L., Heikenwalder M., Kurrer M. O., Behnke S., Frey J., Oxenius A., Joller H., Aguzzi A., Manz M. G., and Speck R. F. / *Proc Natl Acad Sci U S A 2006; 103:15951*-15956
IF: 10.2

158. Simvastatin inhibits the MHC class II pathway of antigen presentation by impairing Ras superfamily GTPases.
Ghittoni R., Napolitani G., Benati D., Ulivieri C., Patrussi L., Laghi Pasini F., Lanzavecchia A., and Baldari C. T. / *Eur J Immunol 2006; 36:2885-2893*
IF: 4.9

159. N-glycan processing in ER quality control.
Ruddock L. W. and Molinari M.
J Cell Sci 2006; 119:4373-4380
IF: 6.5

2007

160. Cyclophilin A, TRIM5, and resistance to human immunodeficiency virus type 1 infection.
Luban J. / *J Virol 2007; 81:1054-1061*
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161. Distinct expression pattern of IFN-alpha and TNF-alpha in juvenile idiopathic arthritis synovial tissue.
Gattorno M., Chicha L., Gregorio A., Ferlito F., Rossi F., Jarrossay D., Lanzavecchia A., Martini A., and Manz M. G. / *Rheumatology (Oxford) 2007; 46:657-665*
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162. Human pregnancy-associated malaria-specific B cells target polymorphic, conformational epitopes in VAR2CSA.
Barfod L., Bernasconi N. L., Dahlback M., Jarrossay D., Andersen P. H., Salanti A., Ofori M. F., Turner L., Resende M., Nielsen M. A., Theander T. G., Sallusto F., Lanzavecchia A., and Hviid L. / *Mol Microbiol 2007; 63:335-347*
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163. The Retroviral Restriction Factor TRIM5alpha.
Sebastian S. and Luban J. / *Curr Infect Dis Rep 2007; 9:167-173*
IF N/A

164. Flt3 in regulation of type-I interferon producing and dendritic cell development.
Onai N., Obata-Onai A., Schmid M. A., and Manz M. G. / *Ann NY Acad Sci 2007;*
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165. Bcl10 controls TCR- and FcgammaR-induced actin polymerization.
Rueda D., Gaide O., Ho L., Lewkowicz E., Niedergang F., Hailfinger S., Rebeaud F., Guzzardi M., Conne B., Thelen M., Delon J., Ferch U., Mak T.W., Ruland J., Schwaller J., and Thome M.
J Immunol 2007; 178:4373-4384
IF: 6.4

166. The role of lysine 186 in HIV-1 integrase multimerization.
Berthoux L., Sebastian S., Muesing M. A., and Luban J. / *Virology 2007; 364:227-236*
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167. Duration, combination and timing: the signal integration model of dendritic cell activation.
Macagno A., Napolitani G., Lanzavecchia A., and Sallusto F. / *Trends Immunol 2007; 28:227-233*
IF: 10.2

168. Lymphocyte activation.
von Andrian U. H. and Sallusto F. / *Curr Opin Immunol 2007; 19:247-248*
IF: 9.1

169. Toll-like receptors and innate immunity in B-cell activation and antibody responses.
Lanzavecchia A. and Sallusto F. / *Curr Opin Immunol 2007; 19:268-274*
IF: 9.1

170. A hypomorphic R229Q Rag2 mouse mutant recapitulates human Omenn syndrome.
Marrella V., Poliani P. L., Casati A., Rucci F., Frascoli L., Gougeon M. L., Lemercier B., Bosticardo M., Ravanini M., Battaglia M., Roncarolo M. G., Cavazzana-Calvo M., Facchetti F., Notarangelo L. D., Vezzoni P., Grassi F., and Villa A.
J Clin Invest 2007; 117:1260-1269
IF: 15.1

171. Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells.
Acosta-Rodriguez E.V., Rivino L., Geginat J., Jarrossay D., Gattorno M., Lanzavecchia A., Sallusto F., and Napolitani G. / *Nat Immunol 2007; 8:639-646*
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172. Glycoprotein folding and the role of EDEM1, EDEM2 and EDEM3 in degradation of folding-defective glycoproteins.
Olivari S. and Molinari M. / *FEBS Lett 2007; 581:3658-3664*
IF: 3.4

173. N-glycan structure dictates extension of protein folding or onset of disposal.
Molinari M. / *Nat Chem Biol 2007; 3:313-320*
IF: 14.5

174. Human-Hemato-Lymphoid-System Mice: Opportunities and Challenges.
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