Scientific Report 2007-2009

Institute for Research in Biomedicine

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This Scientific Report covers the research activities
of the Institute for Research in Biomedicine (IRB)
from July 2007 until June 2009

The report can also be accessed at the IRB's website www.irb.ch

Foreword by Giorgio Noseda
President of the Foundation Council

The period from 2007 to 2009 has seen the further development of the Institute for Research in Biomedicine (IRB) in Bellinzona. The continued and increasing recognition of the excellence of the IRB research and educational programs has led to a long term vision of the institute, housed in a state-of-the-art facility at the heart of a biology campus in Bellinzona as an integral part of the Swiss academic system. The strategy to achieve this future vision is being implemented on local, state and federal levels.

On the federal level, a Memorandum of Understanding has been signed with the Swiss Polytechnic institute in Zurich (ETH). This agreement establishes the grounds for the participation of IRB researchers in the ETH's newly formed Human Health program, the training of ETH Masters and PhD students at the IRB and the creation of a chaired professorship at the ETH for the Director of the IRB, Antonio Lanzavecchia. This professorship will be supported by an annual contribution of the Helmut Horten Foundation. The IRB will also have privileged access to services of the ETH and, in particular, will rely on the ETH Transfer office to better protect and manage IRB intellectual property.

The activities of the Swiss Vaccine Research Institute have begun in 2008. Founded in 2007 together with the CHUV (University Hospital of Canton Vaud), the EPFL (Federal Polytechnic Institute of Lausanne) and the Ludwig Institute and financed by the Bill and Melinda Gates Foundation and the Confederation, the SVRI is funding two programs at the IRB: "Computer Assisted Vaccine Design" by Luca Varani and "Human Cytomegalovirus: from Humoral Immunity to Vaccine Design" by Annalisa Macagno.

On the local, cantonal level, the collaborative project in the field of Computational Biology between the IRB, the University of Italian Switzerland (USI), the Italian Swiss Institute for Oncology (IOSI) and the Swiss National Supercomputing Center (CSCS) is proceeding into its second stage. An affiliation agreement has been signed with the USI and was approved by the governing boards of both institutions. The Cantonal office of university studies is currently preparing a message for approval by the Ticino Parliament (Gran Consiglio) during the summer of 2009. This affiliation will strengthen the Ticino university system and the scientific potential of the canton. The IRB will maintain its independence while increasing the stability and predictability of the public financial contributions it receives. The faculty of the USI, and in particular the newly formed Institute for Computational Studies, will benefit from strong synergies with the IRB. As an officially recognized affiliate of the University, the IRB will postulate formal recognition of its excellent PhD program and will be eligible for federal funding as a recognized academic institution.

The growth of the institute in the past few years has led to the need for new space, a need shared by the IOSI who are currently sharing space with the IRB in both the via Vela and via Murate buildings. Other institutes in the canton have also expressed the need for laboratory space. To meet this need for growth, the City of Bellinzona has identified a construction site

in front of the Espocentro on which the IRB could build a new center housing up to 160 researchers. To assess the feasibility and the costs associated with this ambitious but necessary strategic step, the IRB contracted Architect Mario Campi to study the requirements of such a building and to come up with a preliminary design. Campi was chosen for his experience in the building of the new ETH laboratories in Höngg. The financial feasibility of the project is now being studied and will rely on both public and private funding.
In 2008 the Foundation Council welcomed Prof. Hans Hengartner as a member. Hengartner, currently a member of the IRB Scientific Advisory Board, is Professor Emeritus at the University and Polytechnic of Zurich and is a member of the Board of the Helmut Horten Foundation.
I would like to thank all our faithful supporters and in particular the Helmut Horten Foundation and the Gustav and Ruth Jacob Foundation.

Prof. Dr. med. Giorgio Noseda
Bellinzona, July 2009

Foreword by Antonio Lanzavecchia
Director

This report provides an overview covering the last two years of activity at the Institute for Research in Biomedicine (IRB), and gives insight into ongoing research projects. Currently the IRB hosts nine research groups led by Fabio Grassi, Antonio Lanzavecchia, Markus G Manz, Maurizio Molinari, Silvia Monticelli, Federica Sallusto, Marcus Thelen, Mariagrazia Uguccioni and Luca Varani.
Luca Varani joined the Institute as a group leader in 2007 from Stanford University. Luca has a background in structural biology and is developing new tools to analyze antigen-antibody interactions by combining experimental NMR studies with high throughput computational modeling in order to understand the basis of viral neutralization in dengue and influenza infection. This program is supported by grants from the Cantone Ticino to promote cooperation with the Swiss National Supercomputing Center (CSCS) and from the Swiss Vaccine Research Institute (SVRI), a virtual institute of which IRB is a founding member together with the CHUV, the EPFL and the Ludwig Institute.
During the current period three IRB group leaders were appointed to top-level academic positions at Swiss Universities. Jeremy Luban moved to the University of Geneva where he is Professor of Microbiology and is continuing his research on innate mechanisms of resistance to HIV-1. Maurizio Molinari has been appointed to be an adjunct professor at EPFL. Markus Manz, currently IRB vice director, has recently accepted a chair as Professor of Hematology at the University of Zurich. He has been awarded in 2009 with the San Salvatore prize for his research on stem cells and cancer. We congratulate Markus for his achievements and contributions to the success of the IRB and we are sure that, in his new capacity, he will be able to develop a strong translational program in hemato-oncology at the University of Zurich.
The IRB continues to play a role in education by training PhD students enrolled in Swiss and Italian Universities. IRB students benefit from formal lectures by group leaders, regular journal clubs and a world-class PhD lecture course supported by the Gustav & Ruth Jacob Foundation. PhD students remain the driving force of the IRB and enjoy the open and friendly atmosphere. At present, 31 graduate students work at IRB and 30 have completed their training. Fresh doctorates as well as senior postdocs leaving the IRB have secured excellent positions in leading Institutions in Canada, Germany, Italy, Japan, Portugal, Switzerland and the United Kingdom.
The IRB is internationally recognized as a center of competence in human immunology. The technology to make human monoclonal antibodies by immortalizing human memory B cells has been validated in multiple projects on infectious diseases and has been licensed to a startup company, Humabs, that has established a laboratory in Bellinzona. Two additional platforms have been recently developed. The first is based on long term *in vitro* culture of human plasma cells and is suitable for the selection of specific monoclonal antibodies. The

second can be used to detect and isolate antigen specific T cells from naïve and memory repertoires. A further technology developed at the IRB is the reconstitution of a functional human immune system in a mouse, enabling the study of the human response in a convenient experimental model.

Leukocyte traffic in the immune response remains a major interest of IRB scientists. Recent studies revealed a novel pathway of pathogenic T cell migration into the brain in a model of human multiple sclerosis and delineated a novel subset of T cells devoted to skin immunity. At the molecular level, chemokines have been shown to interact in a synergistic fashion on selected chemokine receptors, a fact that expands our knowledge of the regulation of cell migration during inflammation. Dendritic cell maturation represents a central aspect in the initiation of the immune response. Current research focuses on the role of microbial products, as well as endogenous mediators such as prostaglandins and ATP. Blockade of receptors for ATP promotes T cell anergy both *in vitro* and *in vivo* and blocks the onset of autoimmune diseases in animal models. A new field of research is focused on the role of microRNA in the physiology of mast cells and effector T cells. Threats may also arise from inside the cells when misfolded proteins are not properly disposed. Continuous progress has been made to elucidate the mechanism of protein quality control while antibody fragments have been tested in animal models of Alzheimer's disease for their capacity to prevent the formation of plaques and delay cognitive decline.

The research of the IRB groups is summarized here and is the subject of 55 scientific publications which are listed at the end of this report. IRB scientists have established an effective network of collaborations with leading institutions in Europe, America and Asia. The large number of grants received from the Swiss National Science Foundation, the European Union, the Bill & Melinda Gates Foundation, the Wellcome Trust and the US National Institutes of Health also attest to their success. The last two years have seen a further improvement of our core facilities with the opening of a new animal house, including a BSL3 laboratory, the acquisition of a confocal microscope and equipment for high throughput screening. After ten years of activity from its foundation, I am pleased to say the IRB has gone far beyond the initial expectations and is now an internationally visible center for basic and translational research.

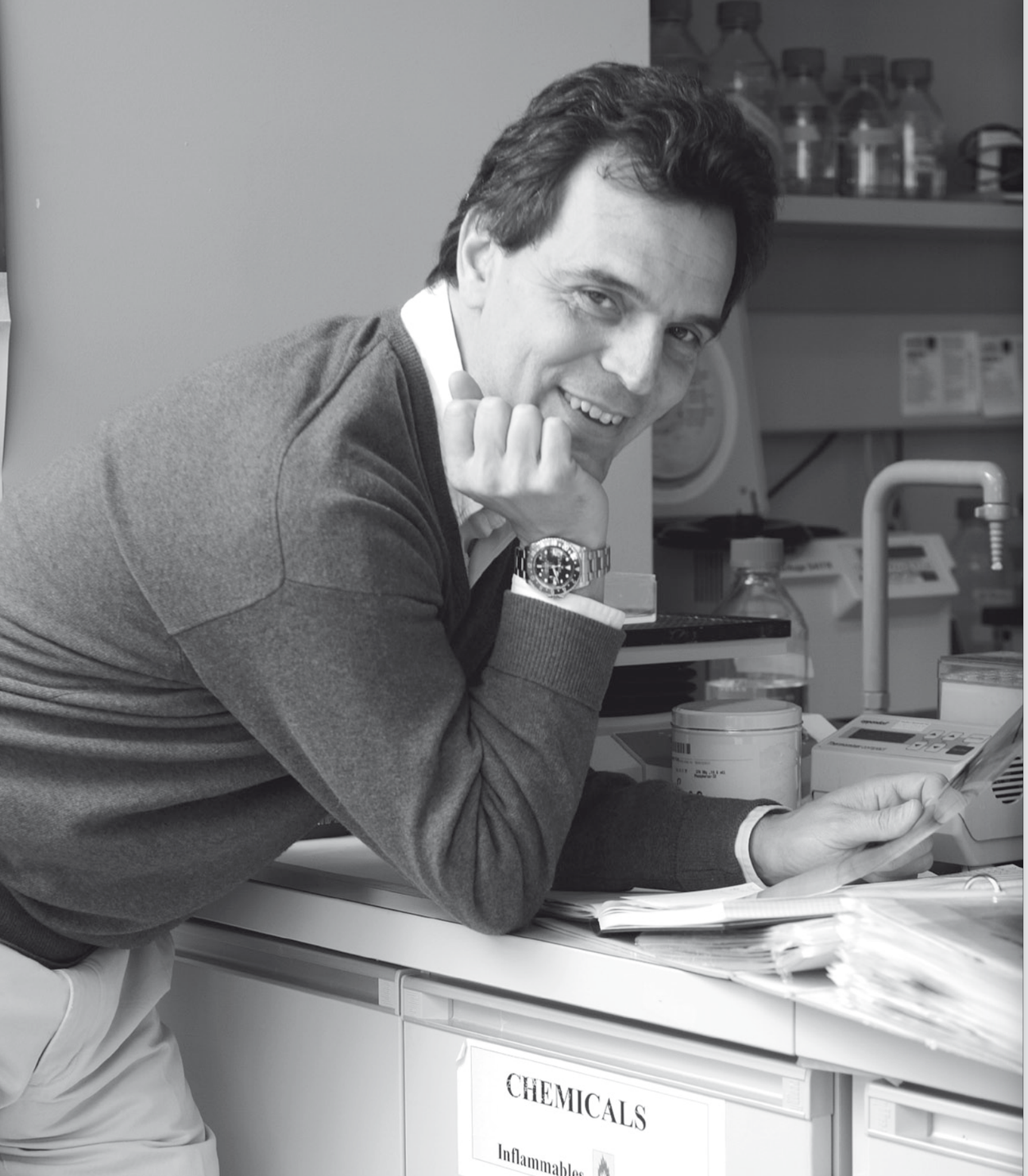
The Institute is especially fortunate to receive core funding from its main sponsors, the Helmut Horten Foundation, the City of Bellinzona, the Cantone Ticino and the Swiss Confederation. Our gratitude also goes to the many individuals who support us through donations and fellowships. We believe that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

Prof. Dr. med. Antonio Lanzavecchia
Bellinzona, July 2009

SECTION 1
RESEARCH PROJECTS

INDEX SECTION 1 - RESEARCH PROJECTS

Fabio Grassi
T Cell Development



Fabio Grassi

Fabio Grassi earned his degree in Medicine at the University of Pavia in 1985 and a Ph.D. in Microbiology at the University of Milan in 1993. He has been Anna Villa Rusconi fellow at the University of Umeä in Sweden (1988), post-doctoral fellow at the Institut Pasteur in Paris (1989-1993), assistant professor at San Raffaele Scientific Institute in Milan (1994-1998), Marie Curie fellow at Hôpital Necker in Paris (1998-2000) and Special Fellow of the Leukemia & Lymphoma Society at Dana Farber Cancer Institute, Harvard Medical School in Boston (2000-2002). 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 Laboratory. His research is focused on various aspect of T cell physiology, including protein and membrane trafficking, signal transduction, control of cell growth and intercellular communications during T cell development in the thymus and in secondary lymphoid organs in immuno-pathological conditions

Research Focus

The experiments performed in the lab are concerned with T cell biology and signal transduction pathways regulating T cell development in the thymus as well as pathophysiological responses in secondary lymphoid organs. Aims of the lab are to characterize T cell lineage commitment and T cell/epithelium cross-talk in the thymus. The knowledge and skills acquired in the developmental biology of the thymus are principally transferred to the study of a rare congenital immunodeficiency, namely Omenn syndrome, in which hypomorphic recombination defects in lymphocytes result in severe thymic atrophy and autoimmunity. Relevant topics addressed in the lab at the moment are to characterize signaling pathways controlled by extracellular ATP in T cell lineage commitment in the thymus as well as in peripheral $CD4^+$ T cell differentiation and function. Apart from antigen presenting cells, the modulation of cellular functions by ATP has been poorly investigated in other cells of the immune system. In T cells we found that during capacitative entry of Ca^{2+} ions, which follows TCR triggering, mitochondrial Ca^{2+} buffering stimulates massive ATP synthesis and release. Pericellular ATP regulates T cell responsiveness through purinergic signaling. The importance of this ATP in regulating T cell function is underscored by the dramatic clinical and histological amelioration of several experimental models of T cell mediated autoimmune diseases by *in vivo* ATP antagonism.

Team

Group Leader: Fabio Grassi, MD, PhD > *fabio.grassi@irb.unisi.ch*
Members: Anna Casati, *PhD student* – Denise Ferrera, *PhD student* – Michela Frascoli, *PhD student* – Michele Proietti, *MD* – Ursula Schenk, *PhD* – Isabella Scheu, *PhD student* – Stefano Volpi, *MD*

Signaling microdomains and molecular determinants in thymocyte β selection

Denise Ferrera and Fabio Grassi

Pre-TCR and γδTCR expression during T cell development define commitment to the αβ and γδ T cell differentiation pathway, respectively. The pre-TCR was shown to transduce the signal for thymocyte β selection in a ligand-independent fashion; whether lipid rafts and pre-TCR affinity for rafts could contribute to the efficiency of signaling at the β selection checkpoint is controversial. In recombinase-deficient thymocyte, calnexin (CNX) associated with CD3 chains is inefficiently retained in the endoplasmic reticulum and weakly expressed in the plasma membrane. Cross-linking of CNX/CD3 reproduces signal transduction by the pre-TCR and phenocopies *in vivo* pre-TCR driven thymus development and thymocyte-epithelium cross talk in RAG-deficient mice. We have shown that analogously to pre-TCR pTα chain, surface CNX is palmitoylated and that CD3 prominently accumulated into lipid rafts upon cross-linking. The mutation of CNX juxtamembrane cysteines with consequent loss of palmitoylation abolished translocation of unretained CNX/CD3 to rafts and rendered surface CNX/CD3 incompetent to signaling. In contrast, introduction of palmitoylated tailless CNX (devoid of ER retention) into recombinase-deficient cells efficiently segregated CD3 into rafts and promoted β selection without any deliberate cross-linking. This implicates lifetime in the plasma membrane of signaling competent complexes and interaction of rafts segregated protein clusters as important elements influencing T cell development. Indeed, the low expression and rapid turnover of endogenous CNX/CD3 in the plasma membrane would impede CD3 accumulation into rafts and illegitimate β selection. Conversely, stable expression of tailless CNX/CD3 in the plasma membrane and targeting into rafts determine pre-TCR-like signaling. These results indicate that rafts might contribute as repositories of protein clusters important in promoting β selection of thymocyte and/or that local thickening of raft membranes or the curvature of the raft membrane might facilitate CD3 accessibility to juxtaposed, palmitoylated tyrosine kinase Lck. Inclusion of palmitoylated cytoplasmic tail from pTα chain in recombinant CNX strikingly improved pre-TCR-like signaling efficiency of CNX/CD3 into rafts. Collectively, our study indicates that lipid rafts in the plasma membrane represent proficient microdomains for initiation of pre-TCR signaling and supports the view that β selection by oligomerized pre-TCR is implemented by pTα cytoplasmic tail.

» ***Ferrera D, et al.***
Eur J Immunol. 2008; 38:1148-1156.

T cell lineage determination by purinergic regulation of TCR signal strength

Michela Frascoli and Fabio Grassi

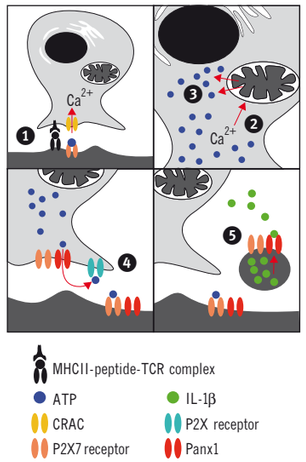
A detailed analysis of transcriptional regulation of purinergic receptors on sorted $CD4^-8^-$ double negative (DN) thymocyte subsets revealed the prominent downregulation of P2X4, P2X7 and P2Y14 receptors upon commitment to the αβ lineage and acquisition of the $CD4^+8^+$ double positive (DP) phenotype. In contrast, P2X7 was prominently expressed in DN cells upon productive rearrangement of γδTCR and commitment to the γδ lineage. Stimulation with 1 mM ATP led to a cytosolic Ca^{2+} rise in a significant percentage of sorted $CD4^-8^-25^+44^-$ double negative 3 (DN3) thymocytes (in which productive TCR gene rearrangement determines lineage commitment), when Ca^{2+} was present in the extracellular medium. ATP triggered also a moderate cytosolic Ca^{2+} rise, due to IP_3 mediated Ca^{2+} release from the ER stores when applied in the absence of extracellular Ca^{2+}, thus confirming the presence of metabotrophic P2Y receptor(s). Using P2X subtype preferring agonists we found that DN3 cells were activated following stimulation with BzATP, a P2X7 specific agonist and UDP-Glucose, a P2Y14 agonist. Therefore, the P2 receptor subtypes detected by RT-PCR were also functionally competent in DN3 thymocytes. In peripheral T cells, we showed that P2X activation by ATP released through pannexin-1 hemichannels upon TCR stimulation represents a crucial autocrine co-stimulus to sustain mitogen activated protein kinase (MAPK) activation and productive T cell activation. In fact, pharmacological antagonism of P2X receptors activity resulted in MAPK inhibition with profound suppression of T cell effector functions. We reasoned that differential regulation of P2X expression during T cell lineage commitment could impact on MAPK signalling. Analogously to mature T cells, albeit at a reduced pace, we showed that DN3 cells released ATP upon CD3 oligomerization. Since αβ versus γδ lineage choice depends on weak versus strong TCR signal strength, respectively, we hypothesized that pharmacologic antagonism of P2X could impact on T cell lineage commitment. We tested this hypothesis in fetal thymus organ cultures (FTOC) and co-culture of DN3 cells with the stromel cell line OP9 transfected with Notch ligand DL-1 (an *in vitro* system that reproduce T cell development). These experiments revealed the crucial role of P2X signalling in determining T cell lineage choice. The P2X antagonist oxidized ATP (oATP) diverted immature $CD25^+$ γδTCR expressing cells toward αβ lineage and determined their aberrant transition to the DP and CD4 single positive stage. We are currently studying the biochemical events underlying this phenomenon. Since ATP is considered a danger associated molecular pattern (DAMP) the diversion of gdTCR expressing cells to the αβ lineage by ATP antagonism implies that γδ T cell development depends more on an innate stimulus than αβ T cells. Accordingly, γδ T cells are placed at the border between the more evolutionarily primitive and rapidly responding innate immune system and the adaptive immune system, where αβ T cells more slowly respond to a given antigen but lead to long-lasting specific memory.

ATP as an autocrine costimulus in T cell activation

Ursula Schenk and Fabio Grassi

» ***Schenk U, et al.*** *Sci Signal. 2008; 1:ra6.*

T cell activation is accompanied by capacitative calcium entry (CCE) through calcium release activated calcium (CRAC) channels in the plasma membrane. Mitochondrial calcium uptake during CCE decreases the local Ca^{2+} concentration near CRAC channels and retards their inactivation. In addition, mitochondrial Ca^{2+} buffering stimulates the oxidative phosphorylation pathway. The efficiency of mitochondrial buffering of Ca^{2+} varies depending on the amount of Ca^{2+} that enters the cell and thus depends on the strength of TCR activation. Weak activation of the TCR induces small elevations in the concentration of cytosolic Ca^{2+}, low uptake of Ca^{2+} by mitochondria and low quantities of ATP synthesized. In contrast, productive T cell activation induces ATP synthesis exceeding the cellular energy demand. We found that ATP is released from efficiently stimulated T cells through pannexin-1 hemichannels and it activated the purinergic P2X receptor (P2XR) to sustain mitogen-activated protein kinase (MAPK) signaling. Because the opening of pannexin hemichannels is regulated by elevations in cytosolic Ca^{2+}, weak TCR stimuli would result in inefficient release of ATP. Our findings suggest that ATP in $CD4^+$ T cells acts as a costimulatory factor whose generation and release depends directly on the strength of T cell activation. P2XR antagonists, such as oxidized ATP (oATP), blunted MAPK activation in stimulated T cells, but did not affect the nuclear translocation of nuclear factor of activated T cells (NFAT), thus promoting T cell anergy. *In vivo* administration of oATP blocked the onset of diabetes mediated by anti-islet TCR transgenic T cells and impaired the development of colitogenic T cells in inflammatory bowel disease. Thus, pharmacological inhibition of ATP release and signaling could be beneficial in treating T cell-mediated inflammatory diseases. Our results point to a selective role of purinergic signaling in sustaining T cell activation and led us to propose a model of T cell activation depicted in the figure on the left. Triggering of the TCR leads to Ca^{2+} influx through CRAC channels (1); Ca^{2+} buffering by mitochondria (2) results in ATP synthesis (3) and the release of ATP through pannexin-1 hemichannels; pericellular ATP activates P2X receptors on the T cell in an autocrine fashion (4) and perhaps P2X7 receptors on the APC in a paracrine fashion resulting in IL-1β processing and release (5).



←*Purinergic control of T cell activation.* *Schematic representation of a T cell (red) interacting with a professional APC (grey). The early phase of T cell activation, which is triggered by the interaction of the TCR with MHC:peptide complex, is characterized by CCE (1), which is accompanied by mitochondrial uptake of Ca^{2+} (2) and Ca^{2+}-dependent stimulation of ATP synthesis (3). ATP is released from activated T cells through pannexin hemichannels, which open when the cytosolic $[Ca^{2+}]$ is elevated. This mechanism guarantees a high concentration of pericellular ATP, which by binding to P2X receptors, serves as an indispensable autocrine costimulus for productive T cell activation (4). Finally, ATP released from activated T cells might also modulate APC function, such as secretion of IL-1 (5).*

Increased ATP production and release by the T cell in the pathogenesis of experimental SLE

Ursula Schenk, Stefano Volpi and Fabio Grassi

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease, characterized by the expansion of autoreactive T lymphocytes. SLE T cells promote polyclonal B cell activation, which leads to hypergammaglobulinemia and organ injury due to immuno complexes deposition. Lupus-prone NZB/NZW F_1 mice displayed expansion of effector/memory T cells compared to Balb/c and C57BL/6 mice and a significant increase in the frequency of T cells secreting pro-inflammatory cytokines *ex vivo*. The molecular mechanisms responsible for increased responsiveness of effector/memory T cells in SLE are not completely elucidated. One feature of autoreactive T cells in SLE is their resistance to activation-induced apoptosis and anergy, which are both connected with an altered mitochondrial metabolism. We observed that responsiveness to TCR stimulation of CD4 effector/memory T cells and, to a lesser extent, of naive T cells isolated from common laboratory strains, were directly correlated to their mitochondrial transmembrane potential under resting conditions. This increased responsiveness might be due to enhanced ATP synthesis and release during activation, which implements autocrine, purinergic costimulation of the T cell. Since T lymphocytes of SLE patients exhibit mitochondrial hyperpolarization, we asked whether this might imply enhanced purinergic T cell costimulation contributing to SLE pathogenesis. Indeed mithocondrial Ca^{2+} uptake was enhanced in effector/memory T cells isolated from NZB/NZW F_1 mice compared to common laboratory strains. Concomitantly, ATP synthesis and release were increased in stimulated effector/memory T cells. *In vitro* treatment of T cells isolated from NZB/NZW F_1 mice with oxidized ATP (oATP) as a purinergic P2X receptors antagonist inhibited T cell activation and induced T cell unresponsiveness. *In vivo* administration of oATP significantly diminished the percentage of effector/memory T cells in secondary lymphoid organs, upregulated anergy-related genes, dramatically ameliorated kidney histopathology and normalized protein concentration in urines of NZB/NZW F_1 mice. These results suggest that effector/memory T cells in NZB/NZW F_1 mice have an altered mitochondrial Ca^{2+} homeostasis, which results in enhanced ATP synthesis and release. Enhanced autocrine purinergic co-stimulation upon TCR triggering would determine peripheral T cell hyper-responsiveness and expansion of effector/memory T cells. Pharmacological ATP antagonism significantly ameliorated immunopathology in NZB/NZW F_1 mice and thus might represent a therapeutic strategy to control SLE-associated glomerulonephritis.

This project is done in collaboration with Elisabetta Traggiai, Istituto G. Gaslini, Genoa (IT)

Regulatory T cell generation by ATP antagonism

Ursula Schenk and Fabio Grassi

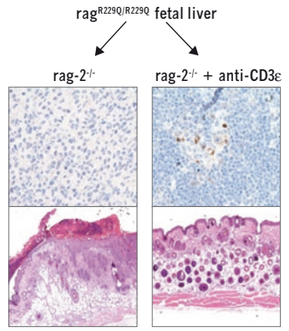
We have shown that inhibition of purinergic P2X receptor signaling in activated T cells by oxidized ATP (oATP) induces a transcriptional program characteristic of anergy. Stimulation of naive $CD4^+$ T cells, together with T cell depleted, irradiated splenocytes in medium containing anti-CD3 antibodies and supplemented with oATP and IL-2, significantly enhanced the percentage of $CD4^+CD25^{high}Foxp3^+$ Treg cells. Analysis of master transcription factors for Th1 (T-bet), Th17 (RORγT) and Treg (Foxp3) lineages by quantitative RT-PCR revealed the progressive upregulation of Foxp3 in the presence of oATP, as opposed to the progressive upregulation of T-bet in untreated cultures. Furthermore, stimulation of sorted $CD4^+CD25^{high}$ cells comprising natural Treg cells induced the expansion of Treg cells with higher expression levels of Foxp3 when oATP was added to the culture medium. In T cells stimulated by anti-CD3 under Th17 skewing conditions (TGF-β and IL-6 conditioned medium) the absolute number of $CD4^+CD25^{high}$ cells expressing Foxp3 was augmented by oATP. The presence of oATP gradually increased the expression of Foxp3 while suppressing the expression of RORγT. A number of soluble mediators were shown to skew *in vitro* differentiation of $CD4^+$ naive T cells toward the Treg lineage, as assessed by Foxp3 expression. However, Treg cells expanded *in vitro* often loose their suppressive phenotype and convert into potentially pathogenic Th17 cells. Addition of oATP to culture medium prevented the conversion of sorted $CD4^+CD25^{high}$ natural Treg cells to the Th17 lineage following stimulation. Contemporaneous injection of oATP with CD4 naive T cells to induce inflammatory bowel disease in lymphopenic mice completely prevented the development of inflammation and promoted the differentiation of adaptive Treg cells. Spleen and mesenteric lymph nodes were not increased and normal numbers of effector/memory T cells in mesenteric lymph nodes were detected. Notably, the effector/Treg cell ratio was significantly increased with respect to mice injected with naive CD4 cells together with Treg cells as healthy controls, suggesting that the presence of oATP at the very initial phase of T cell stimulation efficiently skewed T cell differentiation toward the Treg lineage thus preventing tissue destruction.

Thymic microanatomy in the pathogenesis of Omenn syndrome

Anna Casati and Fabio Grassi

Omenn syndrome (OS) is a combined immunodeficiency due to hypomorphic defects in recombinase activity associated with generalized erythrodermia, alopecia, lymphadenopathy, hepatosplenomegaly, and intractable diarrhea. Oligoclonal T cells infiltrate target organs, suggesting that tolerance to self is compromised in OS. The developmental progression from immature to mature thymocytes occurs sequentially in distinct thymic microenvironments. Lack of appropriate inductive signals from developing thymocytes prevents the formation of distinct cortical and medullary thymic compartments, thus compromising establishment of self-tolerance. Medullary thymic epithelial cells (mTEC) play a pivotal role in self-tolerance induction by determining deletion of autoreactive T cells and promoting maturation of regulatory T cells (Tregs). A subset of mTEC has been shown to express Aire, a transcription factor that controls expression of a variety of tissue-restricted antigens (TRAs) in thymic epithelial cells. These cells express the tight-junctions proteins Claudin-3 and 4 and a yet undefined ligand for the lectin Ulex Europaeus Agglutinin 1 (UEA-1). The thymus from OS patients lacks cortico-medullary demarcation, Hassall's corpuscles and is severely lymphopenic. It displayed barely detectable Aire and TRA transcripts (insulin, cytochrome P450 and fatty acid binding protein). The OS phenotype was reproduced in mice by introducing the hypomorphic *RAG-2* R229Q mutation into RAG2 gene. To see whether altered thymic architecture plays a role in OS pathogenesis we have induced mTEC development in $RAG\text{-}2^{-/-}$ mice by injection of CD3ε specific antibodies, a treatment that implements thymocyte/epithelial cross-talk mediated by pre-TCR expression, which is missing in $RAG\text{-}2^{-/-}$ mice. One week after anti-CD3ε administration, mice were transplanted with E13 fetal liver progenitors from $rag2^{R229Q/R229Q}$ embryos and 8 weeks after transplantation, they were analyzed to evaluate T cell differentiation and immunopathology. In contrast to $rag2^{R229Q/R229Q}$ mice and fetal liver chimeras in $RAG\text{-}2^{-/-}$ mice, fetal liver chimeras in $RAG\text{-}2^{-/-}$ mice conditioned with anti-CD3 antibodies displayed Claudin-4 and Aire expression in the thymus and dramatically reduced percentages of effector/memory T cells in peripheral lymphoid organs. Moreover, significant amelioration of skin histopathology was detected. These results indicate that altered thymic architecture could be at least partially responsible for the severe immunopathology observed in OS patients and warrant consideration of anti-CD3 antibodies as possible treatment in this rare disease.

This project is done in collaboration with Anna Villa, Clinical Institute Humanitas, Milan and Pietro Luigi Poliani, University of Brescia (IT)



➜ ***Aire expression in the thymus and absence of skin lesions in $rag2^{R229Q/R229Q}$ fetal liver chimeras in anti-CD3 conditioned $Rag\text{-}2^{-/-}$ mice.*** *Staining with Aire specific Ig on thymus sections from $rag2^{R229Q/R229Q}$ FLC in $Rag\text{-}2^{-/-}$ mice either injected with PBS (upper left panel) or anti-CD3ε antibodies (upper right panel); Hematoxylin and eosin staining of skin sections from the same mice showing a desquamative lesion with epidermal hyperplasia in FLC in $Rag\text{-}2^{-/-}$ mice injected with PBS (lower left panel) and normal aspect of the skin in FLC in $Rag\text{-}2^{-/-}$ mice injected with anti-CD3ε antibodies (lower right panel).*

Lymphopoiesis in chronic inflammation

Anna Casati and Fabio Grassi

Inflammation promotes granulopoiesis over B lymphopoiesis in the bone marrow (BM). We showed that despite the severe depletion of B cell progenitors during chronic, peripheral T cell-mediated inflammation, the population of mature recirculating B cells was unaffected. These B cells are poised to differentiate to plasma cells in response to blood borne pathogens, in an analogous fashion to non-recirculating marginal zone (MZ) B cells in the

» ***Marrella V, et al.*** *J Clin Invest. 2007; 117:1260-1269.*

» ***Marrella V, et al.*** *Trends Immunol. 2008; 29:133-140.*

spleen. MZ B cells nevertheless differentiated more efficiently to plasma cells upon polyclonal stimulation by Toll-like receptor (TLR) ligands, and were depleted during chronic T cell mediated inflammation *in vivo*. The preservation of mature B cells in the BM was associated with increased concentration of macrophage migration inhibitory factor (MIF) in serum and bone marrow plasma. MIF produced by perivascular dendritic cells in the BM provides a crucial survival signal for recirculating B cells, and mice treated with a MIF receptor antagonist during inflammation showed significantly reduced mature B cells in the BM. We present a model whereby T cell mediated inflammation could impact on the MZ B cell potential to generate T-independent responses against blood-borne pathogens. This possible vulnerability is counteracted by the MIF-mediated preservation of the recirculating mature B cell pool in the bone marrow, thus ensuring serological responsiveness to blood-borne microbes. One possible drawback in the dominant responsiveness to blood-borne antigens by BM mature, recirculating B cells may originate from the distinct repertoire of this subset with respect to MZ B cells. Indeed, the selection of cells lacking N regions and with shorter CDR3s in the immunoglobulin repertoire of MZ B cells but not BM mature B cells was hypothesized to confer to MZ B cells a reduced potential for harmful autoreactivity. Accordingly, TdT deficiency was shown to reduce autoimmune disease incidence and severity. The preferential responsiveness of BM recirculating B cells to blood-borne pathogens thus may impose an autoimmune hazard to the organism during chronic inflammation.

This project is a collaboration with Elisabetta Traggiai, G. Gaslini Institute, Genoa (IT).

Haematopoiesis in T cell mediated inflammation

Anna Casati and Fabio Grassi

Activation and repression of specific genetic programs determine lineage commitment and differentiation of haematopoietic stem cell (HSCs). In pathological conditions perturbation of the homeostasis between cells of different lineages or increased demand of particular subset(s) could modify steady state haematopoietic programs. In two models of T cell mediated inflammation we observed significant increases in HSCs. Relative representation of granulocyte/monocyte progenitors (GMPs) was prominent whereas megakaryocyte/erythroid progenitors (MEPs) and common lymphoid progenitors (CLPs) were dramatically depleted. We followed GMP and MEP representation in lymphopenic mice during homeostatic expansion of $CD4^+CD44^+62L^-$ effector/memory T cells in the absence or presence of tissue inflammation. The increase in peripheral $CD4^+CD44^+62L^-$ effector/memory T cells was positively correlated with the representation of GMP compartment in both "uninflamed" (r = 0.55, P = 0.034) and "inflamed" (r = 0.60, P = 0.014) mice; the interaction term was not significant (P = 0.96) indicating that the degree of correlation was not significantly different in "inflamed" and "uninflamed" mice. This result supports a direct influence of activated T cells on granulopoiesis. Remarkably, MEP representation was positively correlated with effector/memory $CD4^+$ T cell increase in "uninflamed" (r = 0.67, P = 0.005) but was negatively correlated in "inflamed" (r = -0.59, P = 0.034) mice; the interaction term was statistically significant (P = 0.01), indicating that the correlation between MEP representation and effector/memory $CD4^+$ T cell was significantly different between the two conditions. This suggests that inflammatory tissue destruction selectively impinge on GMP versus MEP lineage choice. Since CCAAT/enhancer-binding protein (C/EBP) α is crucially involved in CMP to GMP transition and C/EBPβ was recently hypothesized to be required for granulopoiesis in response to infections ("emergency" granulopoiesis), we quantified the corresponding transcripts. C/EBPα displayed an earlier increase with respect to C/EBPβ but both were significantly increased upon development of overt tissue inflammation, showing that T cell mediated tissue destruction results in upregulation of both these transcription factors.

Details from July 2007 until June 2009

Funding

- ***Swiss National Science Foundation***
  Purinergic signaling in T cell physiology
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- ***European Union***
  MUGEN: Integrated functional genomics in mutant mouse models as tools to investigate the complexity of human immunological disease
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- ***Oncosuisse***
  Sinergy between oncogenic Notch and pre-T cell receptor (pre-TCR) signalling microdomains in leukemogenesis
  01933 / 2007-2009
- ***Ticino Foundation for Cancer Research (Fondazione Ticinese per la Ricerca sul Cancro)***
  Synergy between oncogenic Notch and P2 receptors in leukemogenesis
  2006-2008
- ***Ticino Foundation for Transfusion and Transplantation Research (Fondazione per la Ricerca sulla Trasfusione e sui Trapianti)***
  Calcium dependent shaping of T cell activation
  2008
- ***Converge Biotech Inc., Miami, FL (US)***
  Oxidized ATP as a tool to generate and expand regulatory T cells (Tregs) for the treatment of type 1 diabetes
  2009

Collaborations

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  G. Gaslini Institute, Genoa (IT)
- ***Richard Bucala***
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- ***Lucienne Chatenoud***
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- ***Eugenio Scanziani***
  University of Milan (IT)

Publications

- ***A hypomorphic R229Q Rag2 mouse mutant recapitulates human Omenn syndrome.***
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  Trends Immunol. 2008;29:133-140.
- ***Recombinase-deficient T cell development by selective accumulation of CD3 into lipid rafts.***
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- ***Purinergic control of T cell activation by ATP released through pannexin-1 hemichannels.***
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Lectures and Seminars

- ***G. Gaslini Institute***
  Seminar "Signal transduction and T cell fate"
  Genoa (IT) / 06.12.2007
- ***Miller School of Medicine, University of Miami***
  Seminar "ATP signaling in immunopathology"
  Miami, FL (US) / 17.01.2008

- ***4th MUGEN Annual Meeting***
  Athens (GR) / 29.01.2008
- ***Purines 2008***
  Copenhagen (DK) / 30.06.2008
- ***ThymUS***
  San Juan (PR) / 11.11.2008
- ***University of Florence***
  Seminar "Purinergic signaling in T cell physiology"
  Florence (IT) /18.11.2008

Antonio Lanzavecchia
Immune Regulation

Antonio Lanzavecchia

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 IRB 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, Antonio 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 methods for the efficient isolation of human monoclonal antibodies from memory B cells and plasma cells, which have been successfully applied to infectious diseases such as SARS, Influenza, HCMV, Dengue, Malaria and HIV-1.

Research Focus

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 cells 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 compartments. 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.

Team

Group Leader: Antonio Lanzavecchia, MD › *lanzavecchia@irb.unisi.ch*
Members: Afonso Almeida, *PhD* – Nadia Bernasconi, *PhD* – Giosiana Codoni, *PhD student* – Davide Corti, *PhD student* – Giulia Di Lullo, *PhD* – Blanca Fernandez, *Technician* – Isabella Giacchetto, *Technician* – Annalisa Macagno, *PhD* – Andrea Minola, *Technician* – Giorgio Napolitani, *PhD* – Debora Pinna, *PhD student* – Dora Pinto, *PhD student* – Chiara Silacci, *Technician* – Janine Stubbs, *PhD* – Fabrizia Vanzetta, *Technician*

Identification of a gH/gL/UL128-131A complex recognized by monoclonal antibodies that potently neutralize cytomegalovirus infection of endothelial, epithelial and myeloid cells

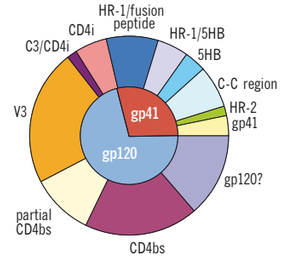
Annalisa Macagno, Nadia Bernasconi, Fabrizia Vanzetta and Antonio Lanzavecchia

Human cytomegalovirus (HCMV) is a widely circulating pathogen that causes severe disease in immunosuppressed adults and upon infection of the fetus. Several glycoprotein complexes are expressed on the surface of HCMV, although gB is thought to be the main target of neutralizing antibodies. Genetic data indicate that UL128, UL130 and UL131 gene products are needed for infection of endothelial, epithelial and myeloid cells. However, antibodies produced by immunization with UL128, UL130 and UL131 proteins showed only poor HCMV neutralizing activity. We therefore decided to take an unbiased approach and to search for antibodies capable of neutralizing infection of either fibroblasts or epithelial and endothelial cells. By immortalizing memory B cells from HCMV-immune donors we isolated several monoclonal antibodies that neutralize at extremely low concentrations (IC90 values ranging from 5 to 200 pM) HCMV infection of endothelial, epithelial and myeloid cells but not of fibroblasts. With a single exception of an antibody that bound a conserved epitope in the UL128 gene product, all antibodies recognized conformational epitopes that required expression of two or more proteins of the gH/gL/UL128-131A complex. Antibodies against gB, gH or gM/gN were also isolated and, albeit less potent, were able to neutralize infection of all target cell tested including fibroblasts. The unusually potent neutralizing antibodies to the gH/gL/UL128-131A complex may find a use not only for passive serotherapy, but also to help the design of immunogens capable of eliciting a potent neutralizing antibody response.

Cross-clade neutralization with coverage of multiple epitopes by human monoclonal antibodies isolated from HIV-1 infected individuals

Davide Corti, Fabrizia Vanzetta, Blanca Fernandez, Chiara Silacci, Debora Pinna and Antonio Lanzavecchia

The isolation and characterization of human monoclonal antibodies capable of neutralizing primary HIV-1 isolates from different clades is a long-desired goal which is instrumental to design an effective HIV-1 vaccine. As part of a consortium effort (CAVD-VDC consortium) we undertook the task of isolating human monoclonal antibodies from non clade B HIV-1 infected donors. We immortalized IgG^+ memory B cells from individuals with broadly neutralizing serum antibodies and screened the culture supernatants using monomeric gp120, trimeric gp140 and gp41 from different clades. This broad approach led to the isolation of 63 monoclonal antibodies that were mapped to different sites of the Env glycoprotein using a variety of experimental approaches including peptide scanning and cross-competition experiments. Several mAbs neutralized isolates of the donor's HIV-1 clade as well as isolates from other clades. In particular, mAbs HK20, HGN194 and HJ16 bound to conserved epitopes in the HR-1, in the V3 crown and in the CD4 binding site and showed considerable breadth of neutralizing activity against a panel of HIV-1 primary isolates spanning both tier 1 and tier 2 viruses of different clades. A cocktail of these three antibodies was able to neutralize all HIV-1 primary isolates tested. The new panel of human monoclonal antibodies described may facilitate the template-based design of immunogens for the development of a vaccine capable of inducing a humoral response against the wide range of HIV-1 strains present in developing countries.



➔ ***Antigenic specificity of 63 human monoclonal antibodies isolated from HIV-1 infected individuals.***

Clonal dissection of the human memory B cell repertoire following infection or vaccination

Debora Pinna, Davide Corti, David Jarrossay and Antonio Lanzavecchia

The analysis of the human memory B cell repertoire is of both fundamental and practical significance. We developed a simple method for the selective activation of memory B cells in total fresh or frozen PBMC using a combination of R848 and IL-2. In these conditions 30-40% of memory B cells generated clones producing on average 200 ng IgG in 10 days. This method was used to measure the frequency of antigen-specific memory B cells as well as the fine specificity, cross-reactivity and neutralizing activity of the secreted antibodies. Following influenza vaccination, specific B cells expanded dramatically, reaching up to 50% of total clonable memory B cells on day 14, and contracted thereafter over several weeks. Specific B cell expansions were detected also in individuals that did not show a significant serological response. Dynamic changes and persistence of B cells specific for a variety of pathogens were documented in serial PBMC samples collected over almost two decades. These results reveal novel aspects of memory B cell kinetics and provide an easy and powerful tool to monitor immune responses following infection or vaccination.

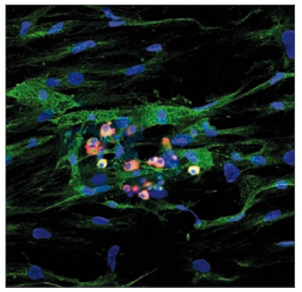
» ***Pinna D, et al.***
Eur J Immunol. 2009;
39:1260-1270

Long term cultures of single plasma cells for the isolation of human monoclonal antibodies

David Jarrossay, Davide Corti, Annalisa Macagno, Giosiana Codoni, Debora Pinna, Markus G Manz and Antonio Lanzavecchia

Plasma cells can survive in appropriate niches of the bone marrow in contact with stromal cells, but die within a few days when put in culture. This has prevented the analysis of plasma cell repertoires for the isolation of human monoclonal antibodies. We hypothesized that bone marrow mesenchymal stromal cells (MSC) might be suitable feeder cells to support plasma cell survival *in vitro*. CD138+ plasma cells isolated from peripheral blood or bone marrow were seeded as single cells on MSC monolayers and IgG production was moni-

tored over several weeks. We found that over this period Ig accumulated in the culture supernatants at a constant rate (70-140 pg/cell/day). IgG and IgA secreting plasma cells were maintained in culture with a plating efficiency ranging from 65% to 100% while the plating efficiency of IgM plasma cells was lower. Ig production was unaffected by hydroxyurea and irradiation as expected for terminally differentiated non dividing cells. We next tested the possibility of using this culture method to select plasma cells secreting antibodies of particular specificities. CD138+ plasma cells were isolated from peripheral blood one week after vaccination or exposure to environmental allergens and cultured on MSC monolayers. The culture supernatants were screened for the presence of specific antibodies using parallel ELISA. From selected cultures the Ig genes were rescued by RT-PCR, inserted in an appropriate vector that was then transfected into 293T cells in order to produce recombinant antibodies. Using this method we were able to isolate a human monoclonal antibody with unusual breadth of reactivity against multiple influenza virus subtypes including both group 1 viruses (H1, H5, H9) and group 2 viruses (H3 and H7). The preservation of human plasma cells in single cell cultures offers, for the first time, the possibility of interrogating plasma cell repertoires in order to isolate rare cells producing antibodies of particular interest and complements the memory B cell immortalization method. Experiments are underway to compare plasma cell and B cell repertoires following vaccination.



← Figure 2. Human plasma cells cultured on mesenchymal stromal cell monolayers.
Red, human IgG. Green, CXCL12 staining.

A broadly neutralizing antibody response to influenza A virus

Davide Corti, Annalisa Macagno, Fabrizia Vanzetta, Giosiana Codoni, Debora Pinna and Antonio Lanzavecchia

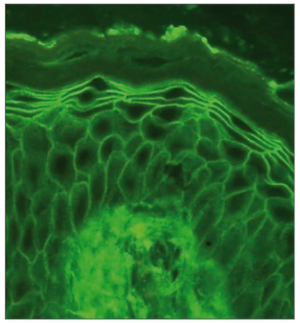
A widely held dogma is that influenza neutralizing antibodies are subtype-specific. Therefore, the identification of antibodies capable of neutralizing multiple subtypes of influenza A virus remains a long-sought goal. Recently two laboratories described antibodies isolated from phage display libraries that neutralize several subtypes belonging to group-1 influenza viruses. To ask whether such antibodies would be also produced in response to influenza vaccination we immortalized IgG+ B cells from individuals that were recently boosted with a seasonal influenza vaccine (containing H1 and H3) and screened the culture supernatants for antibodies that bind to the unrelated H5 hemagglutinin. Using this approach we found that some individuals produce antibodies that bind to H5 and neutralize H5 pseudotypes. In one donor this crossreactive response accounted almost for 30% of the total response to the vaccine. From high responder donors memory B cells and plasma cells were collected at appropriate times after vaccination and broadly neutralizing monoclonal antibodies were isolated by EBV immortalization or single plasma cell culture. From a panel of almost 200 monoclonal antibodies isolated we made the following observations. With only a few exceptions most antibodies bound to an acid sensitive epitope in the stem region, competed with each other and with C179, a previously characterised mouse monoclonal antibody that binds to a conserved epitope in HA stem region. In addition, most of the antibodies used VH1-69 heavy chain variable region while the remaining used different VH. All the antibodies were more effective in pseudotype neutralization rather than virus neutralization. Nonetheless, when tested *in vivo* in an animal model the antibodies were capable of protecting mice from challenge with multiple influenza subtypes (H1, H5, H6 and H9). These antibodies may be useful for passive vaccination and to identify the conserved structures recognized in the view of designing a universal influenza vaccine.

The spectrum of autoantibodies in pemphigus

Giulia Di Lullo and Antonio Lanzavecchia

Pemphigus vulgaris (PV) is a life-threatening autoimmune blistering disease of skin and mucous membranes associated with autoantibodies against the cadherin-type adhesion molecules desmoglein (Dsg)3 and Dsg1. The binding of antibodies to Dsg on epidermal keratinocytes leads to intraepithelial blister formation. At present the involvement of anti-Dsg antibodies in PV pathogenesis is well-established, while the mechanism of blister formation is only partly defined. In addition, autoantibodies against other epithelial antigens have been described, suggesting the occurrence of epitope spreading phenomena. To gain a better insight into PV pathophysiology we immortalized IgG+ B cells from pemphigus patients to isolate the spectrum of autoantibodies characteristic of this disease. As screening assays we used staining of live or fixed and permeabilized keratinocytes as well as ELISA with recombinant proteins. Several clones specific for Dsg1 and Dsg3 were isolated and characterized for their domain specificity and for their capacity to disaggregate keratinocytes. In addition we isolated monoclonal antibodies that bind to the mitotic spindle and to other antigens of unknown origin present on keratinocytes as well as non-epithelial cells. Antibodies that stain keratinocytes were also isolated from normal individuals, but bound exclusively to intracellular antigens. We also collected serial mononuclear cell and serum samples from patients before and at different times after treatment with anti-CD20 antibody (Rituximab) in order to study the dynamics of autoreactive versus vaccine-specific B cells during the B cell depletion and reconstitution phase.

This project is done in collaboration with Giovanna Zambruno and Giovanni Di Zenzo, IDI, Rome, (IT).



➔ Staining of human skin by a monoclonal antibody isolated from a pemphigus patient.

Protective versus pathogenic immune responses to Plasmodium falciparum

Janine Stubbs and Antonio Lanzavecchia

We have taken 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. A high proportion of IgG memory B cells produced antibodies specific for *P. falciparum* antigens and several monoclonal antibodies specific for MSP2 and Pf27 and Pf27A were isolated. These antibodies stain with distinct patterns the surface of merozoites and enhance their killing by human monocytes. Surprisingly, we found that a large fraction of memory B cells produce antibodies that stain hemozoin-associated antigens that are present in the food vacuole and are therefore unlikely to have a protective role. In this respect we have preliminary evidence that hemozoin associated with antibodies might stimulate dendritic cell maturation and that protein and non-protein antigens associated to hemozoin might potently stimulate large number of T cells. Our working hypothesis is that antibodies may play a pathogenetic role in malaria infection by enhancing presentation of hemozoin-associated antigens leading to widespread T cell stimulation and cytokine production that are not beneficial for the host since they are not targeted to the pathogen.

Recovery of the CD8⁺ T cell memory pool after total body irradiation

Afonso Almeida, Federica Sallusto and Antonio Lanzavecchia

Lymphopenia that follows viral infections of therapeutic treatments such as total body irradiation (TBI) is followed by reconstitution of lymphoid compartments within weeks or months. While it is known that T cell numbers recover, the exact contribution of different naive and memory subsets has not been evaluated. To investigate this issue we adoptively transferred $CD8^+$ T cell subsets from mice differing in congenic markers into TBI-treated or T cell deficient hosts and followed their fate during the reconstitution process. We found that in TBI-treated hosts the vast majority of donor-derived cells recovered were the progeny of central memory T cells which appeared to be relatively radioresistant, while naive and effector memory T cells gave only a minor contribution. By studying the early reconstitution kinetics we found that TBI-treated hosts lack fast-proliferating cohorts of naive and effector memory CD8 T cells which were found in T cell deficient mice. This was due to the presence of host radio-resistant $CD4^+CD25^+$ Tregs in TBI-treated animals. We conclude that the selective advantage of central memory T cells is the result of their intrinsic radio-resistance and of the action of $CD4^+CD25^+$ Treg which prevents the expansion of naive and effector memory T cells. The selective expansion of central memory T cells following TBI may skew the memory repertoire and may have influence the response to pathogens and tumors.

This project is done in collaboration with Ilja Ciernik, San Giovanni Hospital, Bellinzona (CH).

On the cellular basis of IgE memory

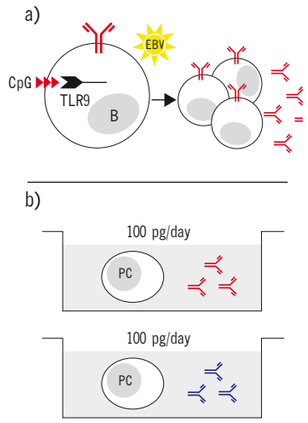
Giosiana Codoni, Giulia Di Lullo, Annalisa Macagno and Antonio Lanzavecchia

While it is well established that allergic individuals mount recall responses to allergens, the basis for such an anamnestic response remains to be elucidated. In particular it is not clear whether surface IgE+ memory B cells exist or whether switch to IgE occurs only during terminal differentiation in target tissues. Several attempts to immortalize B cells sorted according to the expression of membrane IgE led to consistently negative results since the few B cells immortalized were found to be artifacts due to the presence of IgM^+ B cells with rheumatoid factor activity. However, when naive or memory B cells were immortalized with EBV and CpG in the presence of IL-4, a fraction of the growing cells expressed surface IgE. In these conditions IgE^+ clones could be readily isolated by cell sorting and subcloning, indicating that under the aegis of IL-4 naive and memory B cells can switch to IgE and that clones that stably express membrane IgE and secrete IgE can be immortalized. We are therefore considering the possibility that allergen specific memory B cells do not express membrane IgE and have the distinctive capacity to home to allergen exposed sites where they undergo class switch recombination and terminal differentiation under the aegis IL-4 or other switch factors produced *in situ*. The single plasma cell culture method that we developed (see above) offers the opportunity to analyze, for the first time, the repertoire of IgE secreting plasma cells. In preliminary experiments we were able to rescue IgE antibodies from rare IgE-secreting plasma cells. These findings will be extended to the study of allergic individuals exposed to environmental allergens.

Three platforms to interrogate human memory repertoires

Federica Sallusto and Antonio Lanzavecchia

In the last few years our laboratories have developed three technological platforms to interrogate human memory repertoires. These methods allow a comprehensive analysis of the memory response in all its relevant cellular components, namely memory B cells, plasma cells and different subsets of T helper cells. These approaches are facilitated by the miniaturization of the cell cultures and the availability of automatic liquid handling systems combined with high throughput parallel screening methods. For the characterization of neutralizing antibodies we use a variety of approaches including recombinant proteins, infectious viruses and pseudotypes, selection of escape mutants, site-directed mutagenesis and peptide libraries. We plan to integrate these approaches to study the B and T cell response to influenza virus and influenza vaccination.



➔ ***Two methods to isolate human monoclonal antibodies from memory cells.*** **a)** *immortalization of memory B cells using EBV and CpG;* **b)** *long term cultures of plasma cells (PC) followed by screening of the secreted antibodies and rescue of V genes by single cell RT-PCR.*

↘ *See also F. Sallusto report, page 76*

Details from July 2007 until June 2009

Funding

- *Swiss National Science Foundation*
  Role of innate receptors in dendritic cell and lymphocyte activation
  310030-112678 / 2006-2009
- *Swiss National Science Foundation*
  Role of innate receptors in dendritic cell and lymphocyte activation
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- *Human Frontier Science Program*
  Human immune memory and vaccine responses: high troughput analyses of antibody reportoire and function
  RGP9-2007 / 2007-2010
- *European Union*
  HUMALMAB: Human moncolonal antibodies as tolls for malaria research and therapy
  FP6-LHSP-CT-2006-036838 / 2007-2008
- *European Union*
  IMECS: Identification of mechanisms correlating with susceptibility for avian influenza
  FP7-201169 / 2008-2012
- *European Union*
  PEMPHIGUS: from autoimmunity to disease
  FP7-200515 / 2008-2011
- *European Union*
  MUGEN: Integrated functional genomics in mutant mouse models as tools to investigate the complexity of human immunological diseases
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  FP6-2002-503240 / 2003-2008
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  PTVDC: Poxvirus T cell vaccine discovery consortium
  38599 / 2006-2011
- *Wellcome Trust*
  Generation and characterization of human monoclonal antibodies to highly pathogenic H5N1 viruses
  079864-Z-06-Z / 2006-2009
- *European Union*
  DC-THERA: Dendritic cells for immunotherapies
  FP6-NoE-LSHB-CT-2004-512074 /2005-2009

Collaborations

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- *Cameron Simmons*
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- *Kanta Subbarao*
  National Institute of Allergy and Infectious Diseases, Bethesda (US)
- *Nancy Sullivan*
  Vaccine Research Center, Bethesda (US)
- *John Mascola*
  Vaccine Research Center, Bethesda (US)
- *Robin Weiss*
  University College, London (UK)
- *Giovanna Zambruno*
  IDI, Rome (IT)

Publications

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Book chapters

Martin-Fontecha A, Lanzavecchia A, Sallusto F."Dendritic cell migration to peripheral lymph nodes". In Handbook of Experimental Pharmacology. Lombardi G and Riffo-Vacquez Y Eds. Springer, Berlin Heidelberg. 2009

Lectures and Seminars

- *Cambridge Immunology Forum*
  Cambridge (UK) / 27.09.07
- *PVTDC Discovery & Immunology Cores Meeting*
  NewYork, NY (US) / 03.10.07
- *XVth International Immunotherapy Symposia Series Meeting*
  NewYork, NY (US) / 04.10.07
- *EPFL*
  Seminar"Understanding immunological memory: insights into rational vaccine"
  Lausanne (CH) / 12.10.07
- *15th Annual Meeting of the International Cytokine Society*
  San Francisco, CA (US) / 28.10.07
- *7° Congresso Nazionale SIDAPA*
  Modena (IT) / 09.11.07
- *Symposium*
  "Immunity, Immunopathology and Immunoregulation: from antigen presentation to effector mechanisms"
  Rome (IT) / 24.11.07
- *Novartis Pharma AG (NIBR)*
  Seminar"An efficient method to make human monoclonal antibodies against infectious agents"
  Basel (CH) / 22.01.08
- *Vaccinology Course of the Institut Pasteur School of Infectiology*
  Lecture"Immunological memory: the challenge of conferring long-term protection?"
  Paris (FR) / 05.03.08
- *IAVI – R&D Retreat*
  NewYork, NY (US) / 13.03.08
- *Biosymposia - Tregs and Th17 Cells in Autoimmunity*
  Washington, DC (US) / 26.03.08
- *International Menarini Foundation - Respiratory Allergy & COPD from Innate Immunity to Treatment*
  Genoa (IT) / 11.04.08
- *IAVI – NAC Neutralizing Antibody Consortium Annual Meeting*
  La Jolla, CA (US) / 28.04.08
- *Henry Kunkel Society Annual Meeting*
  Santa Margherita Ligure (IT) / 22.05.08
- *6th National Conference SIICA*
  Rome (IT) / 14.06.08
- *B Cell Conference*
  Oxford (UK) / 30.06.08

- *Stem Cell Meeting*
  Lugano (CH) / 02.07.2008
- *Bill & Melinda Gates Foundation*
  Seattle, WA (US) / 28.07.2008
- *2nd Gaslini Advanced Course in Basic and Applied Immunology*
  Keynote Lecture
  Genoa (IT) / 25.09.2008
- *The 10th International Symposium on Dendritic Cells*
  Co-chair"
  Kobe (JP) / 03.10.2008
- *2nd International Conference on Dengue and Dengue Hemorrhagic Fever*
  Phuket (TH) / 10.2008
- *The Scripps Research Institute*
  Seminar"Isolation and characterization of human monoclonal antibodies"
  Jupiter, FL (US) / 13.11.2008
- *Symposium in Honor of Rafi's 60th Birthday*
  Atlanta, GA (US) / 13-16.11.2008
- *MalEra Vaccines Consultative Meeting*
  Montreux (FR) / 22.11.2008
- *Midwinter Conference of Immunologists Dunn-Campbell Lecture*
  Asilomar, CA (US) / 24.01.2009
- *USGEB Annual Meeting*
  STSBC symposium chair
  Interlaken (CH) / 29.01.2009
- *Vaccinology Course – Institute Pasteur School of Infectiology*
  Lecture"Immunological memory: the challenge of conferring long-term protection?"
  Paris (FR) / 09.03.2009
- *Ita Askonas Seminar Series*
  "Understanding and making use of immunological memory"
  London (UK) / 19.03.2009
- *World Immune Regulation Meeting (WIRM) III*
  Davos (CH) / 22.03.2009
- *Lymphocyte, Memory and Diseases – A meeting to celebrate the life and work of Peter Beverley*
  Oxford (UK) / 24.03.2009
- *Keystone Symposium on Antibodies and Drugs*
  Whistler, BC (CA) / 31.03.2009
- *EURO-PADnet Consortium Meeting*
  Rome (IT)/ 4.04.2009
- *Yale University*
  Janeway Memorial Lecture"Dissecting the immune response to pathogens"
  New Haven, CT (US) / 09.04.2009

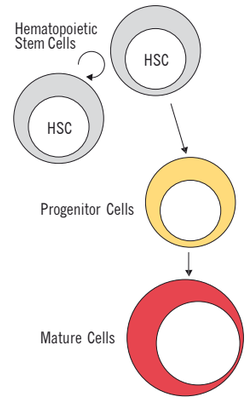
Markus G Manz
Hematopoiesis

Markus G Manz

Markus G Manz received his degree as M.D. in 1995 at the Eberhard-Karls-University Medical School in Tuebingen, Germany, where he also finished his thesis work in 1996 at the Department for Transplantation Immunology. Between 1995 and 1999, he trained in Internal Medicine at Tuebingen University. From 1999 to 2001 he worked as a postdoctoral fellow in the laboratory of Irving Weissman at Stanford, US. In 2002 he became Head of the Hematopoiesis Laboratory at the IRB. From 2004 to 2006 he finished his hematogy/oncology training and board qualification at the University of Tuebingen, while at the same time he worked as associated Group Leader at the IRB. Since September 2006 he is Group Leader at the IRB and attending hematologist at the Oncology Institute of Southern Switzerland (IOSI), Bellinzona. In 2008 he became vice-director of IRB. Markus G Manz received the Artur-Pappenheim Award of the German Society of Hematology and Oncology 2004, and the Fondazione San Salvatore Award 2008.

Research Focus

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 is so far the most successfully working clinical stem cell therapy, applied since more than 50 years for the treatment of malignant hematologic disease or immunodeficiencies. In addition, hematopoietic stem cells currently provide the major gateway for clinical gene therapy. The hierarchically structured 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 hematopoietic stem cell turn-over and hemato-lymphopoid differentiation in physiologic steady-state conditions as well as in inflammation and neoplasia in both mice and men. In depth understanding of 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.



➜ ***Hematopoietic stem cells (HSCs) self-renew and give rise to all mature hematopoietic cells for the life of an individual.*** *We are studying the mechanisms that guide HSC self renewal and allow demand adapted production of mature hematopoietic cells.*

Team

Group Leader: Markus G Manz, MD › *markus.manz@irb.unisi.ch*

Members: Sekhar Boddupalli, *PhD student* – Chiara Borsotti, *PhD student* – Daniela Bossi, *PhD* – Dior Kingston, *PhD student* – Rouven Mueller, *MD* – Michael Schmid, *PhD student* – Hitoshi Takizawa, *PhD* – Patrick Ziegler, *PhD*

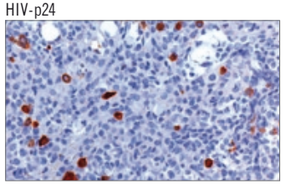
Human hemato-lymphoid-system-mice as preclinical models

Chiara Borsotti, Dior Kingston, Hitoshi Takizawa, Rouven Mueller, Patrick Ziegler and Markus G Manz

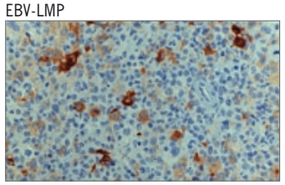
Because ethical and practical restrictions limit *in vivo* studies in humans, small animal models that faithfully resemble human physiologic and pathologic *in vivo* settings are valuable for predictive *in vivo* preclinical testing. Multiple substitute human to small animal xenotransplantation models have been employed. Existing models, however, sustain 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 gamma-irradiation conditioned newborn immunodeficient $Rag2^{-/-}\gamma c^{-/-}$ mice leads to *de novo* development of human B, T, natural interferon-producing cells (also called plasmacytoid DCs) and dendritic cells (DCs); formation of structured primary and secondary lymphoid organs; and production of some functional immune responses. Using this model, we are currently studying human hematopoietic cell development and maintenance, infection, immune responses, and therapeutic interventions in human hemato-lymphoid system specific virus infections, and engraftment, differentiation and therapy of human malignant hematopoietic cells. As long-term human HSC maintenance, human myeloid differentiation, and adaptive immune cell development and function is still limited in current $Rag2^{-/-}\gamma c^{-/-}$ and other immunodeficient mice, further improvement for faithful resemblance of the human hemato-lymphoid-system in this small animal model is warranted. In a collaborative, Gates Foundation Grant Challenges in Global Health Program supported project we are thus replacing a selection of mouse cytokines and MHC components with human counterparts.

This project is a collaboration with the laboratory of R. Flavell, Yale University, and S. Stevens, Regeneron (US).

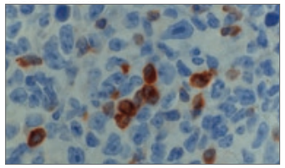
HIV-p24



EBV-LMP



T Cell-Perforine



←Human specific viral infection in Human-Hemato-Lymphoid System Mice. *Human-Hemato-Lymphoid-System $Rag2^{-/-}\gamma c^{-/-}$ mice can be infected with human specific lymphotropic viruses and show some adaptive immune responses. Lymph node sections showing immunohistochemistry for productive HIV infection (upper panel, anti-HIV p24 stain), EBV infection (middle panel, anti-EBV-LMP stain), and EBV infected lymph node infiltrating T cells expressing perforine (lower panel, anti-perforine stain).*

Hematopoietic stem cell population dynamics

Hitoshi Takizawa, Sekhar Boddupalli, Rouven Mueller and Markus G Manz

Most data on HSCs has been gained in mice. Mouse HSCs can be prospectively isolated to about almost purity, and it has been estimated that an adult mouse contains about 15,000 HSCs. A minor fraction of these circulate in blood at any given time of which some re-home to marrow HSC niches and continue to give rise to blood cells for the lifetime of a mouse. Adult mouse HSC numbers are kept fairly constant by homeostatic maintenance, i.e. slow cell turn-over, while *in vivo* HSC expansion occurs prenatally, postnatally until adulthood, and after *in vivo* ablation and transplantation of limited numbers of HSCs. We recently developed a method to track single HSCs and their divisional status *in vivo* over several

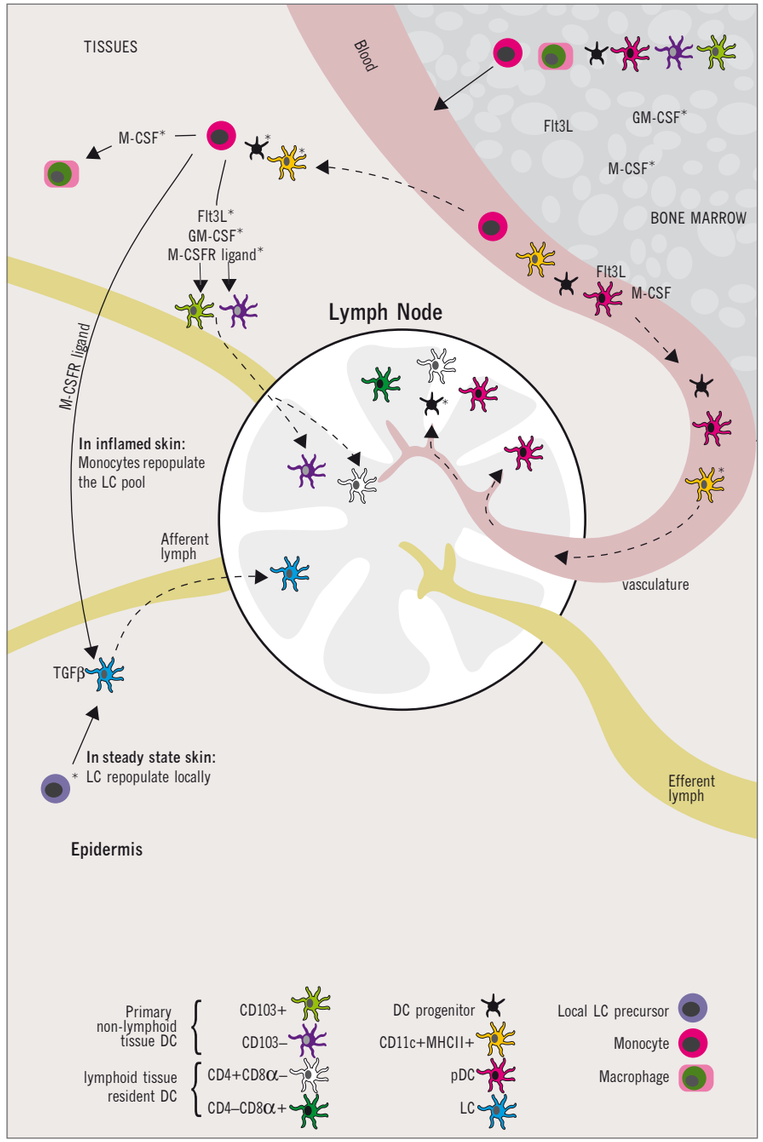
months in mice and are currently addressing several fundamental open questions in HSC biology: a) what signals regulate HSC pool size and homeostasis?; b) how many HSCs of the total HSC pool contribute to hematopoiesis at any given time?; c) does HSC division occur in fixed time-intervals and what is the relation between divisional history and contribution to hematopoiesis?; d) is HSC division influenced by hematopoietic challenges as severe infection?; and e) how does divisional history and HSC aging correlate with malignant HSC transformation? Clarifying these issues will help to better understand evolution of HSC alterations causing clinical disease and ultimately to develop more appropriate therapies.

Dendritic cell homeostasis

Dior Kingston, Sekhar Boddupalli, Michael Schmid and Markus G Manz

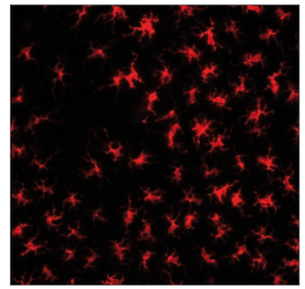
Dendritic cells (DCs) are rare hematopoietic cells that co-evolved with the formation of the adaptive immune system. DCs efficiently process and present antigen, move from sites of antigen uptake to sites of cellular interactions, and are critical in the initiation of immune responses as well as in the maintenance of self-tolerance. DCs are distributed throughout the body and are enriched in lymphoid organs and environmental contact sites. Steady-state DC half-lives account for days to up to few weeks and they need to be replaced via proliferating hematopoietic progenitors, monocytes, or tissue resident cells. DCs are a heterogeneous population of cells that can be divided into two major populations: (1) nonlymphoid tissue migratory and lymphoid tissue resident DCs and (2) plasmacytoid DCs (pDCs, also called natural interferon-producing cells, IPCs). An important focus of our laboratory is to study the physiology of DC homeostasis. Our working hypothesis is that DC development is regulated via positive and/or negative feedback mechanisms (as e.g. erythropoiesis or thrombopoieis) at the hematopoiesis-immune system interface to ensure appropriate DC regeneration upon demand in steady-state and inflammation. Over the last several years, we were able to demonstrate that pDC and DC differentiation potential is confined to $Flt3^+$-tyrosine kinase expressing hematopoietic progenitor cells, that over-expression of human *Flt3* in $Flt3^-$- and $Flt3^+$-progenitors and consecutive enforced Flt3-signaling induced transcription of pDC-, DC-development affiliated genes and rescued and enhanced their pDC and DC differentiation potential, respectively. We also were able to identify pDC and DC development restricted $Flt3^+$ progenitors (clonal common dendritic cell committed progenitors, CDPs) in bone marrow, and demonstrated that they are major, if not exclusive contributors to steady-state DC homeostasis. Pharmaceutical inhibition of Flt3 signaling caused a reversible *in vivo* pDC and DC deficiency that resembled the deficiency observed in Flt3-ligand knockout mice. Together, these findings suggest an environmental Flt3-ligand cytokine driven model, where Flt3 acts as the earliest inducer and a constant enhancer of steady-state IPC and DC generation. Interestingly, inhibition of Flt3 signaling as well as depletion of DCs caused an *in vivo* increase of Flt3-ligand, suggesting a regulatory feedback loop tailored to sustain constant pDC and DC levels. We are now addressing regulation of DC differentiation by studying DC development relevant cytokine/cytokine receptor expres-

» ***Kingston D, et al.***
Blood. 2009;
113:3418-3427



sion and cytokine production in steady-state and immunological challenges, by testing the influence of inflammatory stimuli on DC development, and by interfering pharmacologically with development and evaluating resulting impact on *in vivo* immune responses. 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.

←***Dendritic cell homeostasis: HSCs produce DC progenitors, pDCs and DCs in the BM.*** *Flt3 ligand is a non-redundant cytokine for BM DC differentiation, while the exact role of GM-CSF and M-CSFR ligands remains to be determined. BM -derived circulating blood cells maintain, with the exception of epidermal LCs, all known steady-state DC homeostasis in lymphoid and non-lymphoid tissues. We hypothesize that progenitor cells with limited proliferation potential upon Flt3 ligand and LTβ stimulation enter the lymph nodes (LNs) through HEV to maintain the majority of LN DCs in steady-state. It is also possible that non-proliferating blood DCs follow the same route. In addition, non-lymphoid tissue DCs continuously enter the LNs through afferent lymphatics, but these represent only a minority of steady-state LN DCs. The specific contribution of proliferating DC progenitors, blood DCs, and monocytes to non-lymphoid tissue DCs in the steady-state, and the relative involvement of cytokines as Flt3 ligand, GM-CSF, and M-CSFR ligands, remain to be addressed. In contrast to most DCs, LCs repopulate locally in the steady-state either through self-renewal or through a local hematopoietic precursor that takes residence in the skin. In inflamed skin, monocytes repopulate the LC pool via a TGFβ and MCSFR-dependent pathway. In the steady-state, pDCs are recruited to the LNs and other lymphoid organs directly from the blood and, with the exception of the liver, enter most non-lymphoid tissues only upon inflammation. If lymphoid organ pDCs also derive from DC precursors entering the organs remains to be determined. (*) Likely, but not formally proven. Illustration modified from: Dendritic cell homeostasis. Merad M, Manz MG. Blood. 2009;113:3418-3427.*



➔***Langerhans cells are dendritic cells of the epidermis, i.e. the primary host-environment interface.*** *Picture depicts MHC class II staining of a mouse epidermal sheet from the ear.*

Hematopoieis supporting mesenchymal stromal cells

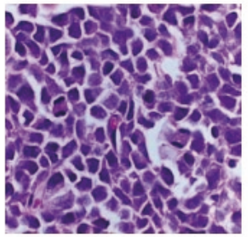
Patrick Ziegler, Steffen Boettcher, Michael Schmid, Hitoshi Takizawa and Markus G Manz

Adult mammalian hemato-lymphopoiesis, with the exception of T cell maturation, is primarily located in the bone marrow and is tightly regulated by constitutive and inducible expression of cytokines and other factors that act on receptor expressing precursor cells and modulate respective hematopoietic lineage cell production. While regulation of red blood cell production via renal erythropoietin release is well understood, the mechanisms that guide myeloid cell generation upon specific demand have not been defined. Because bone marrow stromal cells provide the niches for hematopoietic differentiation and produce stem cell and hemato-lymphoid cell supporting cytokines, we postulate that they are equipped

with sensing mechanisms to detect the need for homeostatic and demand-adapted myelopoiesis requirements. We have found high expression of some pattern recognition receptors on bone marrow stromal cells and demonstrate enhanced production of HSC and myelopoiesis supporting factors upon experimental ligation of these. We are currently evaluating these processes in defined settings *in vivo*.

Myeloproliferative neoplasia initiating cells

Daniela Bossi, Hitoshi Takizawa, Rouven Mueller and Markus G Manz



Chronic myelogenous leukemia (CML) and myeloproliferative neoplasias (MPNs, including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (MF)) are a heterogeneous group of clonal hematological diseases that are characterized by overproduction of differentiated and functional erythro-myeloid cells and/or platelets. While MPNs have been shown to derive from disease initiating cells that reside in the hematopoietic stem and progenitor cell compartment, separation of MPN initiating cells from coexisting healthy HSCs and progenitors has not been achieved. We postulate that one mechanism how MPN initiating cells outcompete healthy HSCs over time, is due to an enhanced MPN initiating cell cycle activity. We thus work on the establishment of an MPN xenogeneic mouse model that will be used for identification and functionally characterization of human MPN initiating cells *in vivo*. This project should provide insights into basic biology of MPNs and might deliver a preclinical tool for targeted MPN initiating cell drug testing *in vivo*.

←***Engraftment of human CML blast-crisis cells in Rag2$^{-/-}\gamma c^{-/-}$ mice.*** *Upper panel depicts HE stain demonstrating multiple mitosis, lower panel shows FACS analysis using anti human CD34 and CD45 antibodies*

Details from July 2007 until June 2009

Funding

- ***Swiss National Science Foundation***
  In vivo evaluation of HIV infection using mice that carry a human adaptive immune system
  3100A0-108352.1 / 2005-2007
- ***Swiss National Science Foundation***
  Regualtion of dendritic cell development from hematopoietic stem and progenitor cells
  310000-116637.1 / 2007-2010
- ***European Union***
  DC-THERA: Dendritic cells for immunotherapies
  FP6 – LSHB-CT-2004-512074 / 2008-2009
- ***European Union***
  STROKEMAP: Multipotent adult progenitor cells to treat stroke
  FP6 - LSHB-CT-2006-037186 / 2006-2009
- ***Bill and Melinda Gates Foundation Challenges in Global Health Program (GC#4)***
  A humanized mouse model to evaluate live attenuated vacchine candidates
  2005-2010
- ***Oncosuisse***
  An *in vivo* study on the stem cell origin of chronic myeloproliferative disease
  OCS-02019-02-2007 / 2008-2010
- ***Helsinn Group***
  Research Support
  2009

Collaborations

- ***Richard Flavell***
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- ***Sean Stevens***
  Regeneron, Terrytown, NY (US)
- ***Miriam Merad***
  Mount Sinai School of Medicine, New York, NY (US)
- ***Tim Sparwasser***
  Twincore, Helmholz-Zentrum and MHH, Hannover (DE)
- ***Tim Bruemmendorf***
  University of Hamburg (DE)
- ***Joachim Hauber***
  Heinrich Pette Institute, Hamburg (DE)
- ***Hongkui Deng***
  University of Peking (CN)
- ***Gerard Bos***
  University of Maastricht (NL)
- ***Catherine Verfraillie***
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- ***Jeremy Luban***
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- ***Nicolaus Kroeger***
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- ***Richard Stanley***
  Albert Einstein College of Medicine, NY (US)
- ***Mark Suter***
  Bavarian Nordic, Munich (DE)
- ***Robert Weissert***
  Merck-Serono, Geneva (CH)

Publications

- ***Identification of clonogenic common Flt3+M-CSFR+ plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow.***
  Onai N, Obata-Onai A, Schmid M A, Ohteki T, Jarrossay D, Manz M G.
  Nat Immunol. 2007;8:1207-1216.
- ***Macrophage tolerance: CD47-SIRP-alpha-mediated signals matter.***
  Takizawa H, Manz M G.
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- *The STATs on dendritic cell development.*
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- *CD34+ cord blood cell-transplanted Rag2$^{-/-}$ gamma(c)$^{-/-}$ mice as a model for Epstein-Barr virus infection.*
  Cocco M, Bellan C, Tussiwand R, Corti D, Traggiai E, Lazzi S, Mannucci S, Bronz L, Palummo N, Ginanneschi C, Tosi P, Lanzavecchia A, Manz M G, Leoncini L.
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- *Human T cell development and HIV infection in human hemato-lymphoid system mice.*
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- *Transient depletion of RUNX1/RUNX1T1 by RNA interference delays tumour formation in vivo.*
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- *Dendritic cell homeostasis.*
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- *Flt3L, DCs, and NTregs: team contra GVHD?*
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- *The concerted action of GM-CSF and Flt3-ligand on in vivo dendritic cell homeostasis.*
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- *Humanized mice for modeling human infectious disease: challenges, progress, and outlook.*
  Legrand N, Ploss A, Balling R, Becker P D, Borsotti C, Brezillon N, Debarry J, de JongY, Deng H, Di Santo J P, Eisenbarth S, Eynon E, Flavell R A, Guzman C A, Huntington N D, Kremsdorf D, Manns M P, Manz M G, Mention J J, Ott M, Rathinam C, Rice C M, Rongvaux A, Stevens S, Spits H, Strick-March-and H, Takizawa H, van Lent A U, Wang C, Weijer K, Willinger T, Ziegler P.
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- *Potent inhibition of HIV-1 by TRIM5-cyclophilin fusion proteins engineered from human components.*
  Neagu MR, Ziegler P, Pertel T, Strambio de Castillia C, Gruetter C, Martinetti G, Mazzucchelli L, Gruetter M, Manz MG, Luban J.
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Book chapters

Manz MG."Dendritic cell and natural type I interferon-producing cell development"in Antigen Presenting Cells and the Eye. Eds. M Zierhut, H G Rammensee and J W Streilein. Informa Healthcare USA, Inc., NewYork, NY 2007

Croy BA, Di Santo JP, Manz MG, Bankert RB. "Mouse models of immunodeficiency"in The Mouse in Biomedical Research. 2nd Edition Volume 4 Immunology. Eds. J G Fox; S W Barthold, M T Davisson, C E Newcomer, F W Quimby and A S Smith. Academic Press Burlington MA. 2007

Ziegler P, Manz MG."Mouse Models for Human Hemato-Lymphopoiesis"in Current Protocols in Toxicology John Wiley & Sons, Inc. 2007

Lectures and Seminars

- *Keystone Symposium on Immunological Intervention in Human Disease*
  Big Sky, MT (US) / 10.01.2007

- *Heidelberger Akademie der Wissenschaften, University of Heidelberg*
  Hematology Lecture"Animal models in stem cell research"
  Heidelberg (DE) / 02.02.2007

- *Heinrich-Pette-Institut*
  Seminar"Human immune system Rag2$^{-/-}\gamma c^{-/-}$ mice: New options to study human lymphotropic viruses *in vivo*"
  Hamburg (DE) / 02.03.2007

- *DCcrest07: DC-THERA Graduate School*
  Celerina (CH) / 30.03.2007

- *The Swiss Society for Allergy and Immunology Annual Meeting and Spring Meeting SGDV-SSDV*
  Basel (CH) / 20.04.2007

- *21st European Immunogenetics and Histocompatibility Conference, European Federation for Immunogenetics*
  Barcelona (ES) / 07.05.2007

- *Annual retreat of the Infection Biology Program at Karolinska Institutet*
  Lecture"Human hemato-lymphoid system mice"
  The Island of Sandhamn, Stockholm (SE) / 30.08.2007

- *Keystone Symposium on Challenges of Global Vaccine Development*
  Cape Town (ZA) / 09.10.2007

- *Childrens Hospital University of Zurich*
  Seminar"Human Immune System Mice"
  Zurich (CH) / 23.10.2007

- *University of Bern*
  Immunology Seminar series "Dendritic cell differentiation and homeostasis"
  Bern (CH) / 31.10.2007

- *The 37th Annual Meeting of the Japanese Society for Immunology*
  Tokyo (JP) / 21.11.2007

- *University of Geneva*
  Biology Seminar series"Dendritic cell development and homeostasis"
  Geneva (CH) / 25.01.2008

- *The Immunology Institute Mount Sinai School of Medicine*
  Seminar"Human Hemato-Lymphoid System Rag2$\gamma c^{-/-}$ mice"
  NewYork, NY (US) / 04.02.2008

- *Virologisches und Immunologisches Kolloquium, University of Würzburg*
  Seminar"Human-Hemato-Lymphoid Sysetm Mice: a preclinical *in vivo* model"
  Würzburg (DE) / 11.02.2008

- *University of Basel*
  DBM-Schwerpunktseminar "From hemato-poietic stem to immune system cells"
  Basel (CH) / 15.02.2008

- *University of Freiburg*
  Hematology Lecture"Humane Hämatopoese im Maus-Modell: Diagnostische und therapeutische Perspektiven"
  Freiburg (DE) / 22.02.2008

- *Weizmann Institute of Science*
  Seminar "hemato-lymphopoiesis: differentiation, homeostasis, and neoplasia"
  Rehovot (IL) / 27.03.2008

- *DC-THERA Annual Meeting*
  Athens (GR) / 10.04.2008

- *Genmab*
  Seminar "Human immune system mice for anti-human mAb Testing"
  Utrecht (NL) / 17.04.2008

- *International Extranodal Lymphoma Study Group Annual Meeting 2008*
  Lugano (CH) / 25.04.2008

- *European Institute of Oncology*
  Seminar "Human immune system mice as preclinical testing Models"
  Milan (IT) / 05.05.2008

- *Henry Kunkel Society Annual Meeting 2008*
  Santa Margherita Ligure (IT) / 24.05.2008

- *Wilsede Meeting*
  Wilsede (DE) / 15.06.2008

- *Laboratory Retreat Prof. Aguzzi Lab University of Zurich*
  Lectures "Human immune system mice", "identification and function of dendritic cell progenitors"and"hematopoietic stem cell dynamics"
  Kloental (CH) / 18.07.2008

- *22nd Annual Meeting European Macrophage and Dendritic Cell Society*
  Brescia (IT) / 18.09.2008

- *10th International Symposium on Dendritic Cells*
  Kobe (JP) / 01.10.2008

- *LyFE organized by Oncology Institute of Southern Switzerland*
  Bellinzona (CH) / 16.10.2008

- *Life Science College Peking University*
  Seminar"Human-hemato-lymphoid-system mice"
  Bejing (CN) / 24.10.2008
- *Weatherall Institute of Molecular Medicine, Oxford*
  Seminar"Homeostatic dynamics of hematopoietic stem cells"
  London (UK) / 04.11.2008
- *King`s College London*
  Seminar"Homeostatic dynamics of hematopoietic stem cells"
  London (UK) / 05.11.2008
- *Helmholtz Zentrum München*
  Hämatologikum Seminar "Divisional dynamics in hematopoietic stem cells"
  Munich (DE) / 27.11.2008
- *Yale Uinversity*
  Hematology Seminar series"Divisional dynamics of hematopoietic stem cells"
  New Haven (CT) / 03.12.2008
- *Workshop on "Monocyte, macrophage and dendritic cell heterogeneity"*
  Treilles (FR) / 06.03.2009
- *Keystone Symposium on Dendritic Cells*
  Banff (CA) / 31.03.2009
- *II International Workshop on Humanized Mice*
  Organizer
  Amsterdam (NL) / 03.04.2009
- *EMBO Conference – Tackling and imaging the complexity of the immune system*
  Capo Caccia (IT) / 22.04.2009
- *Swiss Hematology Association, Annual Meeting*
  Basel (CH) / 15.05.2009
- *Translational Research in Paediatric Rheumatology -TRiPR innate immunity and the pathogenesis of rheumatic diseases*
  Genoa (IT) / 27.05.2009
- *European Hematology Association (EHA) Annual Meeting*
  Berlin (DE) / 05.06. 2009

Maurizio Molinari
Protein Folding and Quality Control

Maurizio Molinari

Maurizio Molinari earned a PhD in Biochemistry at the ETH-Zurich in 1995. In 1996-1997 he was post-doc in the laboratory of Cesare Montecucco at the Department of Biomedicine, University of Padua (IT) and subsequently in the laboratory of Ari Helenius at the ETH-Zurich (1998-2000). Since October 2000, he is Group Leader at the IRB. The studies performed by Molinari's group gave significant contribution to the knowledge of mechanisms devised by cells for 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 the beta-amyloid, a toxic peptide that deposits in the human brain eliciting neurodegenerative processes associated with the Alzheimer's disease. Maurizio Molinari 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. In 2008 he was appointed Adjunct Professor at the EPFL.

Research Focus

About 30% of eukaryotic gene products is synthesized by ribosomes attached at the cytosolic face of the endoplasmic reticulum (ER) membrane. The ER lumen contains resident molecular chaperones and folding factors that assist their maturation. Native proteins are released from the ER and are transported through the secretory pathway to their final intra- or extra-cellular destination. Folding-defective polypeptides are exported across the ER membrane into the cytosol and destroyed (*figure page 55*). Cell and organism homeostasis relies on a balanced activity of the ER folding, quality control and degradation machineries as shown by the dozens of human diseases related to defective maturation or disposal of individual polypeptides generated in the ER. The aim of our work is to understand the molecular mechanisms regulating chaperone-assisted protein folding and protein disposal from the mammalian ER. We are also interested in understanding the regulation of chaperone content in the ER lumen. A thorough knowledge of these processes is essential to learn how to intervene in protein biogenesis. The capacity to manipulate protein folding, quality control and degradation will be instrumental to delay progression or even to cure diseases caused by inefficient functioning of the cellular protein factory. It will also increase productivity of recombinant proteins to be employed in the clinics and in the industry.

Team

Group Leader: Maurizio Molinari, PhD › *maurizio.molinari@irb.unisi.ch*

Members: Riccardo Bernasconi, *PhD student* – Siro Bianchi, *Technician* – Lara Brambilla, *Undegraduate student* – Verena Calanca, *Technician* – Tito Calì, *PhD student* – Christian Caprara, *Undegraduate student* – Carmela Galli, *Research assistant* – Silvia Olivari, *PhD student* – Tatiana Soldà, *Research assistant* – Omar Vanoni, *PhD student*

A role for OS9 variants in protein quality control in the mammalian ER

Riccardo Bernasconi and Maurizio Molinari

Non-native polypeptides are retained in the ER and are dislocated into the cytosol where they are degraded by proteasomes. We have characterized the function in ER quality control of two proteins derived from alternative splicing of the OS9 gene. OS9.1 and OS9.2 are ubiquitously expressed in human tissues and are amplified in tumors. We have shown that OS9.1 and OS9.2 are transcriptionally induced upon activation of the Ire1/Xbp1 ER-stress pathway. They do not associate with folding-competent proteins. Rather, they selectively bind folding-defective ones thereby inhibiting transport of non-native conformers through the secretory pathway. The intralumenal level of OS9.1 and OS9.2 inversely correlates with the fraction of a folding-defective glycoprotein, the $Null_{hong\ kong}$ (NHK) variant of α1-antitrypsin, that escapes retention-based ER quality control. OS9 up-regulation does not affect NHK disposal, but reduction of the intralumenal level of OS9.1 and OS9.2 substantially delays disposal of this model substrate. Our data show that OS9.1 and OS9.2 play a dual role in mammalian ER quality control: firstly as crucial retention factors for misfolded conformers, and secondly as promoters of protein disposal from the ER lumen.

This project was a collaboration with Thomas Pertel and Jeremy Luban (IRB, Bellinzona and the University of Geneva, CH).

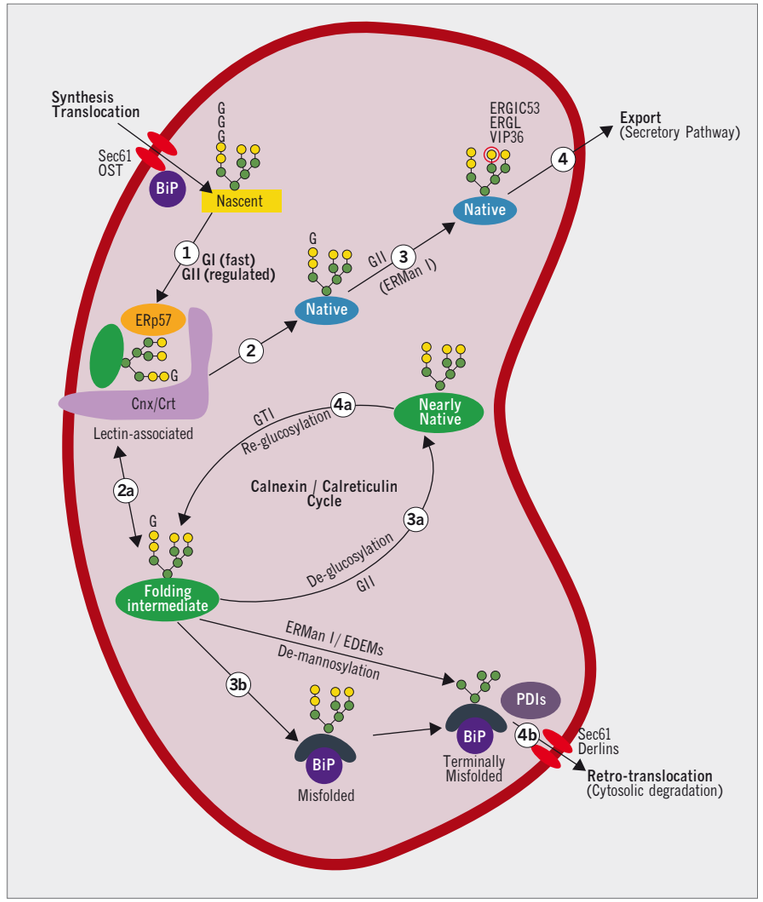
» ***Bernasconi R, et al.*** *J Biol Chem.* 2008; 283:16446-16454.

Segregation and rapid turnover of EDEM1 modulates standard ERAD and folding activities

Tito Calì, Carmela Galli, Silvia Olivari and Maurizio Molinari

EDEM1 is a crucial regulator of ER-associated degradation (ERAD) that extracts non-native glycopolypeptides from the calnexin chaperone system. Under normal growth conditions, the intralumenal level of EDEM1 must be low to prevent premature interruption of ongoing folding programs. Our studies showed that in unstressed cells, EDEM1 is segregated from the bulk ER into LC3-I-coated vesicles and is rapidly degraded. The rapid turnover of EDEM1 is regulated by a novel mechanism that shows similarities but is clearly distinct from macroautophagy. Cells with defective EDEM1 turnover contain unphysiologically high levels of EDEM1, show enhanced ERAD activity and are characterized by impaired capacity to efficiently complete maturation of model glycopolypeptides.

» ***Cali T, et al.*** *Biochem Biophys Res Commun.* 2008; 371:405-410.



synthesized glycoproteins, folding is not completed in a single round of association with Cnx/Crt (step 2a). The folding intermediate released from the lectin chaperones is de-glucosylated (step 3a) but its forward transport is inhibited by GT1. GT1 adds back a glucose residue (step 4a) only to glycoproteins with nearly native conformation. These re-bind to Cnx/Crt and are subjected to additional folding attempts likely to consist in disulfide reshuffling. Glycopolypeptides released from Cnx/Crt and displaying major folding defects are ignored by GT1 (step 3b). Rather, they attract BiP. They are extensively de-mannosylated and dislocated across the ER membrane for proteasome-mediated degradation (step 4b).

Molecular characterization of ERAD tuning

Siro Bianchi and Maurizio Molinari

We define as *ERAD tuning* the *post*translational mechanisms operating in the mammalian ER at steady state to rapidly remove EDEM1 (and other ERAD regulators) from the ER lumen. We propose that *ERAD tuning* is an important, uncharacterized mechanism of regulation of the intracellular content of select ER-resident proteins. It relies on their segregation from the bulk ER, their enrichment in LC3-I-coated vesicles and their degradation by lysosomal enzymes. Aim of this project is the identification of the proteins whose intralumenal level is regulated by *ERAD tuning*, of the mechanisms of segregation of these proteins from the long living chaperones that are retained in the bulk ER and of the mechanisms regulating the vesicular transport out of the ER (which we know shares some analogy, but it is clearly distinct from autophagy). We hypothesize that the vesicular transport out of the ER regulating ERAD tuning could be hijacked by pathogens such as viruses and/or bacteria. A literature survey led us to identify viruses that could potentially use these host cell mechanisms for replication of their genome or for budding. Studies are in progress to verify if and how mouse hepatitis virus exploits the ERAD tuning machinery for replication.

This project is a collaboration with Fulvio Reggiori, University of Utrecht (NL).

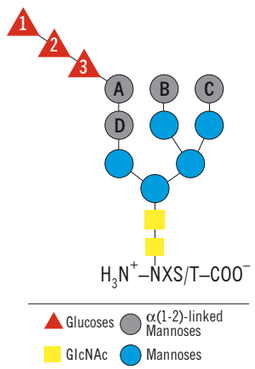
Consequences of N-glycan deletion and proteasomal inhibition on secretion of active BACE

Omar Vanoni and Maurizio Molinari

» ***Vanoni O, et al.*** *Mol Biol Cell.* 2008; 19:4086-4098.

BACE is an aspartic protease involved in the production of amyloid-beta (Aβ), a toxic peptide accumulating in the brain of Alzheimer's disease patients. After attainment of the native structure in the ER, BACE is released into the secretory pathway. In order to better understand the mechanisms regulating protein biogenesis in the mammalian ER, we determined the fate of five variants of soluble BACE with 4, 3, 2, 1 or 0 N-linked glycans. The number of N-glycans displayed on BACE correlated directly with folding and secretion rates and with the yield of active BACE harvested from the cell-culture media. Addition of a single N-glycan was sufficient to recruit the calnexin chaperone system and/or for oligosaccharide de-glucosylation by the ER-resident α-glucosidase II. Addition of 1 to 4 N-glycans progressively enhanced the dissociation rate from BiP and reduced the propensity of newly synthesized BACE to enter aberrant soluble and insoluble aggregates. Finally, inhibition of the proteasome increased the yield of active BACE, as if for BACE the quality control system would be acting too stringently in the ER lumen thus causing loss of functional polypeptides.

This project was a collaboration with Paolo Paganetti, Novartis Pharma, Basel (CH)



←*N-Glycan composition.* *The oligosaccharide added to the nascent polypeptide is composed of two N-acetylglucosamine (yellow squares), nine mannose (blue and grey circles) and three glucose residues (red triangles). Mannose residues linked with an α1,2 bond are shown as grey circles. Removal of these mannose residues by ER-resident mannosidases (e.g. EDEM proteins) results in deviation of the misfolded polypeptide into the ERAD pathway.*

Characterization of Malectin, a novel ER-resident lectin

Riccardo Bernasconi, Verena Calanca and Maurizio Molinari

This project is concerned with a newly discovered ER-resident carbohydrate-binding protein named Malectin. First detected in *Xenopus laevis*, Malectin is highly conserved in animals. Among almost 270 mammalian-type sequences examined by carbohydrate microarray in the lab of our collaborator Ten Feizi, the Xenopus protein showed a unique selectivity of binding to a di-glucosylated high-mannose *N*-glycan sequence: $Glc_2Man_7GlcNAc$. Based on current knowledge, this oligosaccharide structure is very unlikely to be displayed on newly synthesized polypeptides emerging in the ER lumen. To establish the function of Malectin in living cells is therefore of great interest. We prepared vectors for expression of Malectin in mammalian cells as well as cell lines characterized by low content in endogenous Malectin (obtained by stable RNA interference). Consequences of Malectin overexpression and down-regulation on protein biogenesis and quality control are under investigation.

This project is done in collaboration with Ten Feizi, Imperial College London (UK) and Markus Aebi, ETH-Zurich (CH).

Interactomic studies to characterize regulation of protein quality control in the ER lumen

Carmela Galli and Maurizio Molinari

The aim of the project is to understand the rules governing intervention of molecular chaperones, folding enzymes and quality control factors during protein biogenesis in the ER lumen. To this end, we will compare the ER-resident proteins associating with folding-competent proteins *versus* those interacting with folding-defective ones. We will also determine how differences in protein topology (e.g., presence/absence of a transmembrane anchor) or in the glycosylation state (e.g., presence/absence of N-linked oligosaccharides) affect the molecular composition of the model-protein's interactome. We have already generated mouse cell lines that ectopically express, individually, several epitope-tagged model proteins. Immunoisolated model cargo-proteins and their interacting partners will be sent to our collaborator Manfredo Quadroni in Lausanne for analysis. Tryptic fragments will be separated by nano-HPLC followed by tandem MS. Fragmentation spectra for trypsin fragments of proteins will be matched to a mouse protein database sequence with the Mascot software. Specific RNAi will confirm intervention of select "interactors" in polypeptide maturation or disposal.

This project is a collaboration with Manfredo Quadroni, University of Lausanne (CH).

Novel approaches to inhibit production and deposition of the toxic Aβ peptide

Siro Bianchi and Maurizio Molinari

Sequential cleavages of the human amyloid precursor protein (APP) by beta- and gamma-secretases generate the amyloid-beta (Aβ), a 42-residues, aggregation-prone toxic peptide associated with neurodegeneration in Alzheimer's disease. We recently designed vectors for expression in mammalian cells of single chain antibodies, Fab fragments and full length monoclonal antibodies specifically binding residues 3 to 6 of the Aβ peptide. When expressed intracellularly, these molecules associate with newly synthesized APP thus substantially interfering with beta-secretase cleavage and Aβ production (Paganetti et al. 2005). When added extracellularly, they associate with surface exposed APP similarly interfering with Aβ production (unpublished). For studies in an animal model for Alzheimer's disease, we encapsulated stable C2C12 cells producing high levels of the Aβ-directed single chain antibody or Fab fragment in appropriate capillary tubes (capsules) with protein-permeable walls. Capsules implanted in mice brains maintained for several months the capacity to secrete the antibody derivatives, which diffused in the tissue and substantially reduced production and deposition of Aβ. This resulted in a substantial delay of cognitive impairment in this mouse model for Ahzheimer's disease (ongoing).

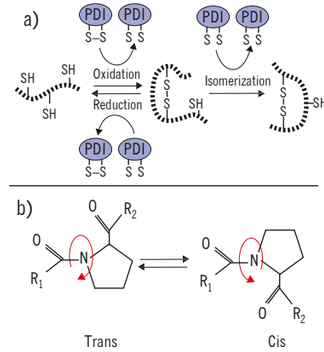
This project is a collaboration with Patrick Aebischer, EPFL, Lausanne (CH)

Cyclophilins intervention in protein biogenesis and quality control in the mammalian ER

Tatiana Soldà, Carmela Galli and Maurizio Molinari

Formation of intra- and inter-molecular disulfide bonds and *cis/trans* isomerization of prolyl bonds are rate-limiting steps for folding of nascent polypeptides. A role of oxidoreductases in polypeptide maturation is well documented, while a role of peptidyl prolyl *cis/trans* isomerases (PPIs) in conformational maturation of nascent polypeptides or in protein quality control in the ER lumen is not supported by experimental data. To study this, we performed preliminary experiments in cells exposed to Cyclosporine A (CsA), a specific inhibitor of immunophilin members of the PPIs family. Cyclophilin A and B (CypA and B) are the major cytosolic and ER-resident targets of CsA, respectively. Our analysis revealed that cells exposed to CsA readily mounted an unfolded protein response characterized by splicing of the Xbp1 transcripts and by transcriptional up-regulation of several UPR markers. CsA-treatment also lowered the intralumenal calcium concentration. Despite these pleiotropic effects of CsA, maturation of influenza virus hemagglutinin, a sensitive marker of variations in the folding capacity in the ER lumen, was not affected upon cell exposure to CsA. CsA did not interfere with degradation from the ER of several membrane-anchored folding-defective polypeptides. Rather, it selectively inhibited disposal from the ER of the same folding-defective polypeptides when not anchored to the ER membrane. Experiments are ongoing to understand if and how CypA or B or other immunophilins play a role in regulation of polypeptide degradation from the ER.

This project is done in collaboration with Thomas Pertel and Jeremy Luban (IRB, Bellinzona and the University of Geneva, CH)



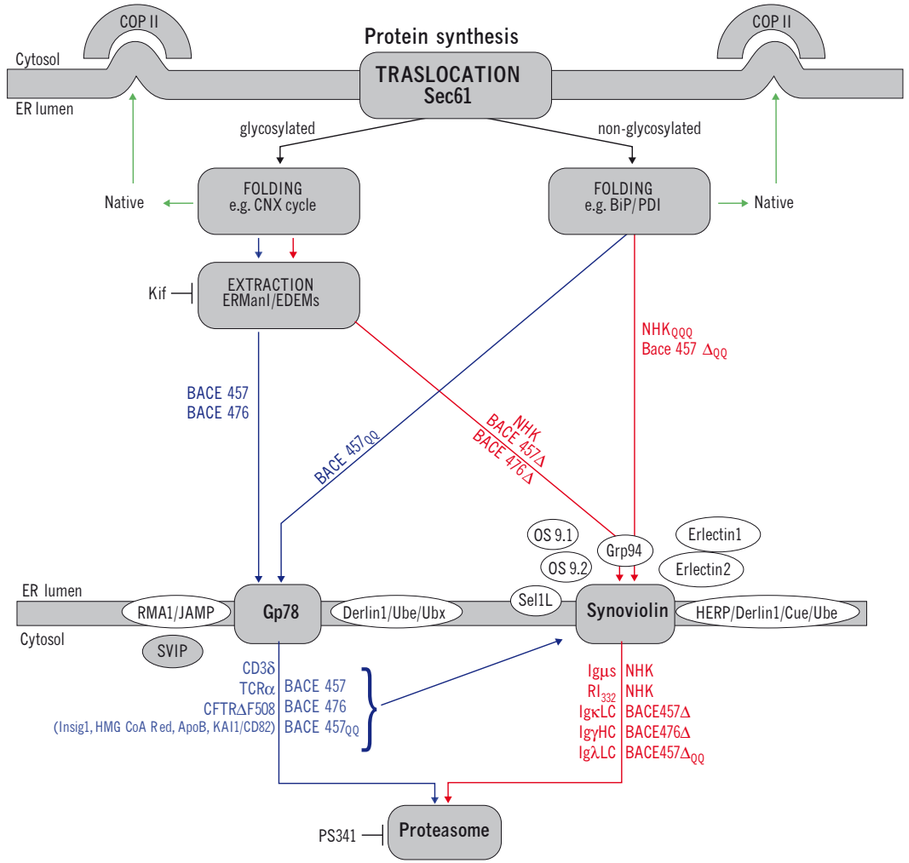
➜ ***The two rate-limiting reactions in protein folding in the ER.*** **a)** *Oxidation, reduction and isomerization of disulfide bonds.* **b)** *Isomerization of peptidyl-prolyl bonds.*

E3 ubiquitin ligases and their involvement in ERAD

Riccardo Bernasconi and Maurizio Molinari

The capacity to rapidly remove folding-defective polypeptides from the ER lumen is crucial to maintain cell homeostasis. Several ER-resident proteins survey maturation of incoming cargo to eventually interrupt unsuccessful folding-attempts and to facilitate dislocation of terminally misfolded polypeptides across the ER membrane for degradation by cytosolic proteasomes. Current models claim that dislocation is regulated by multi-protein complexes built around E3 ubiquitin ligases embedded in the ER membrane. Amongst the several E3 ligases in the mammalian ER membrane (Synoviolin/HRD1, gp78, TEB4, Trc8, RFP2, RMA1) and in the cytosol (e.g. CHIP, Parkin, Fbx2), Synoviolin/HRD1 and gp78 have a documented involvement in disposal of several distinct folding defective polypeptides from the ER. Nevertheless, no information is available on substrate characteristics that determine the selection of the Synoviolin/HRD1 or the gp78-regulated ERAD pathway. The aim of this project is to understand which substrate feature determines the selection of the Synoviolin/HRD1 or of the gp78 pathways for protein disposal from the mammalian ER. To this end, we will investigate the consequences of inactivation of one, the other, or both pathways in cells expressing several extensively studied soluble or membrane-bound ERAD substrates *(figure page 60)*.

This project is a collaboration with Toshihiro Nakajima, St Marianna University School of Medicine, Kawasaki Kanagawa (JP)



↑ ***Complexity and interconnections of quality control pathways offered to cargo proteins emerging in the ER lumen through the Sec61 translocon.*** *Color code serves to distinguish membrane-bound (in blue) from soluble proteins (in red). Proteins analyzed in our lab are in bold. Our preliminary results show that soluble, folding-defective polypeptides preferentially use pathways converging at the Synoviolin complex, while membrane-anchored proteins may use different dislocation complexes.*

Determining EDEM proteins glycanase activity

Riccardo Bernasconi and Maurizio Molinari

EDEM1, EDEM2 and EDEM3 are regulators of glycoprotein disposal from the ER lumen. Some controversy does exist on their function in the ER lumen. In particular, it is disputed if they are mannose-binding lectins or mannose-processing enzymes. As a lectin, EDEM1 would act as a receptor for misfolded polypeptides to be degraded; as a mannosidase, EDEM1 would be the crucial modifier of N-linked oligosaccharides that generates the glycan signal required for extraction of folding-defective proteins from the folding machinery and their deviation in the disposal pathway (*(figure page 55)*. Our studies in living cells showing that EDEM1 is an active mannosidase would support this second hypothesis (Olivari S, et al. 2006). These studies remain controversial because, so far, it has not been possible to confirm the glycanase activity of EDEM1 with purified components in test tubes. The aim of this project is to determine if mammalian EDEM1 and EDEM2, a protein recently characterized in our lab (Olivari et al. 2005) are active mannosidases. To this end, we will express and purify the recombinant proteins and determine their capacity to hydrolyze oligosaccharides of established composition in an *in vitro* assay.

This project is a collaboration with Yukishige Ito, RIKEN Advanced Science Institute, Saitama (JP)

Details from July 2007 until June 2009

Funding

- *Swiss National Science Foundation*
  Protein folding, quality control and degradation in the ER. 3100A0-107578 / 2006-2008
- *Swiss National Science Foundation*
  Protein folding, quality control and degradation in the ER. 3100A0-121926 / 2009-2011
- *Bangerter Foundation / Synapsis Foundation*
  Use of specific antibodies and intrabodies to regulate Aβ production / 2005-2009
- *Foundation for Research on Neurodegenerative Diseases*
  β-secretase as model to investigate the mechanisms of ERAD
  2002-2009
- *NCCR of Competence in Research on Neural Plasticity and Repair*
  Alzheimer's disease
  2001-2009
- *San Salvatore Foundation*
  Protein folding
  2006-2009
- *Swiss Foundation for Aging Research*
  Immunotherapy to reduce the production of the toxic amyloid β peptide
  2007

Collaborations

- *Markus Aebi*
  Swiss Federal Institute of Technology (ETH), Zurich (CH)
- *Fulvio Reggiori*
  University of Utrecht (NL)
- *Manfredo Quadroni*
  University of Lausanne (CH)
- *Ten Feizi*
  Imperial College London (UK)
- *Toshihiro Nakajima*
  St Marianna University School of Medicine, Kawasaki Kanagawa (JP)
- *Yukishige Ito*
  RIKEN Advanced Science Institute, Saitama (JP)
- *Jeremy Luban*
  University of Geneva (CH)
- *Paolo Paganetti*
  Novartis Pharma, Basel (CH)
- *Patrick Aebischer*
  Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne (CH)

Publications

- ***Substrate-specific requirements for UGT1-dependent release from calnexin.***
  Soldà T, Galli C, Kaufman RJ, Molinari M.
  Mol Cell. 2007;27:238-249.
- ***In and out of the ER: protein folding, quality control, degradation, and related human diseases.***
  Hebert D N, Molinari M.
  Physiol Rev. 2007;87:1377-1408.
- ***A dual task for the Xbp1-responsive OS-9 variants in the mammalian endoplasmic reticulum: inhibiting secretion of misfolded protein conformers and enhancing their disposal.***
  Bernasconi R, Pertel T, Luban J, Molinari M.
  J Biol Chem. 2008;283:16446-16454.
- ***Segregation and rapid turnover of EDEM1 by an autophagy-like mechanism modulates standard ERAD and folding activities.***
  Cali T, Galli C, Olivari S, Molinari M.
  Biochem Biophys Res Commun. 2008;371:405-410.
- ***Consequences of individual N-glycan deletions and of proteasomal inhibition on secretion of active BACE.***
  Vanoni O, Paganetti P, Molinari M.
  Mol Biol Cell. 2008;19:4086-4098.
- ***The endoplasmic reticulum crossroads for newly synthesized polypeptide chains.***
  Cali T, Vanoni O, Molinari M.
  Prog Mol Biol Transl Sci. 2008;83:135-179.

Book chapters

Bernasconi, R. and Molinari M."ER-Associated Folding and Degradation: Learning From Yeast?"in Protein misfolding: New Research O'Doherty, C B and Byrne, A C Eds, Nova Science Publishers, Inc., Hauppauge, NY. 2008

Lectures and Seminars

- *Basic Virology Course, Institut Pasteur*
  Lecture"The folding of viral glycoproteins in the endoplasmic reticulum"
  Paris (FR) / 07.09.2007
- *IX Annual Congress Italian Life Sciences Federation (FISV)*
  Riva del Garda (IT) / 23.09.2007
- *Annual Meeting of the Society for Glycobiology*
  Boston, MA (US) / 11.11.2007
- *National Research Council (CNR), Institute for Neuroscience*
  Lecture "ERAD tuning…or the rapid degradation of ERAD regulators"
  Milan (IT) / 01.02.2008
- *University of Bern*
  Joint Seminar series"Protein biogenesis in the mammalian ER"
  Bern (CH) / 25.02.2008
- *31st Annual Meeting of the German Society for Cell Biology*
  Marburg (DE) / 12.03.2008
- *Cold Spring Harbor: Molecular chaperones and stress responses*
  Cold Spring Harbor NY (US) / 30.04.2008
- *NCCR Neural plasticity and repair*
  Zurich (CH) / 02.07.2008
- *Basic Virology Course, Institut Pasteur*
  Lecture"The folding of viral glycoproteins in the endoplasmic reticulum"
  Paris (FR) / 03.09.2008
- *VIII International Workshop on calreticulin and calcium-binding proteins*
  Viña del Mar (CL) / 06.01.2009
- *Annual Meeting of the Union of the Swiss Societies for Experimental Biology (USGEB)*
  Interlaken (CH) / 29.01.2009
- *Forum on Biochemistry of Signal Transduction*
  Milan (IT) / 24.03.2009
- *Forum Alzheimer, Cantonal Psychiatric Clinic*
  Mendrisio (CH) / 30.04.2009
- *First Workshop on "Protein Misfolding in the Test Tube and in Disease"*
  Kibbutz Ein Gedi (IL) / 13.05.2009
- *EMBO Conference Series: The Biology of Molecular Chaperones*
  Dubrovnik (HR) / 23.05.2009
- *FASEB Summer Research Conference: "From Unfolded Proteins in the Endoplasmic Reticulum to Disease"*
  Saxtons River, VT (US) / 07.06.2009
- *Cellular and Molecular Responses to Stress*
  Milan (IT) / 15.06.2009

Silvia Monticelli
Molecular Immunology

Silvia Monticelli

Silvia Monticelli earned a degree in Biology at the University of Milan where she specialized in Molecular Biology. She did her thesis at the San Raffaele Scientific Institute in Milan with Donata Vercelli. 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 (US), and in February 2007 she joined the IRB as Junior Group Leader. Silvia 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.

Research Focus

MicroRNAs (miRNAs) are a family of small non-coding RNAs that have emerged as key post-transcriptional regulators in a wide variety of organisms. Because each miRNA can regulate expression of a distinct set of genes, miRNA expression can shape the repertoire of proteins that are actually expressed during development, differentiation or disease. The generation of the immune system from hematopoietic stem cells involves ordered events of lineage commitment, differentiation, proliferation, and cell migration; within such processes, miRNAs appear ideally suited to rapidly adjust protein concentrations. Accordingly, some miRNAs are expressed in a stage-specific fashion, and miRNA control has recently emerged as a critical regulatory principle in the mammalian immune system. Indeed, genetic ablation of the miRNA machinery, as well as loss or deregulation of certain individual miRNAs, severely compromises immune development and leads to immune disorders like autoimmunity and cancer. In our lab we study the role of miRNAs in the differentiation of cells of the immune system, with a special focus on T lymphocytes and mast cells. Besides being of fundamental relevance to our understanding of cell differentiation and gene regulation, elucidation of the molecular mechanisms that control mast cells and T helper cell differentiation and activation have substantial potential for clinical application in the treatment of asthma, allergy, autoimmunity, chronic inflammation and malignancies.

Team

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Members: Lorenzo Dehò, *PhD student* – Ramon J Mayoral, *PhD student* – Nicole Rusca, *PhD student*

Role of miR-221-222 in murine mast cells differentiation and function

Ramon J Mayoral and Silvia Monticelli

Mast cells (MCs) are cells of the innate immune system that reside in most tissues and derive from hematopoietic precursors in the bone marrow. Mast cells can function as effector and immunoregulatory cells in IgE-associated allergic disorders, as well as in certain innate and adaptive immune responses. We analyzed the expression pattern of several miRNAs in murine MCs differentiated *in vitro*, and we identified a family of two miRNAs, miR-221 and miR-222, that were transcriptionally upregulated upon acute activation of differentiated MCs, pointing towards a potential role for these miRNAs in regulating MC effector functions. To identify potential *cis*-regulatory regions that might give us insights into the mechanisms of miR-221-222 transcriptional regulation in murine MCs, we analyzed the pattern of DNase I hypersensitivity (HS) sites in the miR-221-222 genomic locus, and we identified some conserved, MC-specific, accessible regions upstream the miR-221-222 sequences. Bioinformatics analysis for putative transcription factor binding sites in these regions identified motifs that were over-represented in the DNase I HS sites as compared to promoter sequences in the entire murine genome. Combining such bioinformatics analysis with experimental approaches, we were able to show that the calcineurin/NFAT and IKK/NFκB pathways are important regulators of miR-221-222 expression in MCs, and we predicted a regulatory circuit comprising miR-221-222 and the transcriptional silencer Zbtb16 as potentially relevant for MC differentiation. To investigate the role of miR-221-222 expression in MCs survival, proliferation and functions, we transduced the mast cell line MC/9, as well as primary bone marrow-derived MCs (BMMCs) with lentiviral-based vectors to stably over-express miR-221 and miR-222. Over-expression of either miRNA, but not of a mutant version of miR-221, induced downregulation of the described miR-221-222 targets c-KIT and $p27^{Kip1}$. Most interestingly, over-expression of these miRNAs led to an alteration of the cell cycle profile with reduced cell proliferation. Our working hypothesis is that miR-221-222 might have a dual role in murine mast cells: the first one would be to regulate differentiation from hematopoietic progenitors, possibly in a molecular circuit with Zbtb16. The second function would be to regulate cell activation in differentiated MCs, and more specifically, miR-221-222 expression could help the cell to go back to a resting state after acute activation, by contributing to block, at a post-transcriptional level, expression of genes that belong to the previous, activated state. We are now continuing our analysis of miR-221-222 transcriptional regulation, as well as of their *in vivo* role in MC differentiation and functions by taking advantage of mouse models lacking MCs.

» ***Majoral RJ, et al.*** *J Immunol.* 2009; 182:433-445.

miR-146 and adaptive immunity

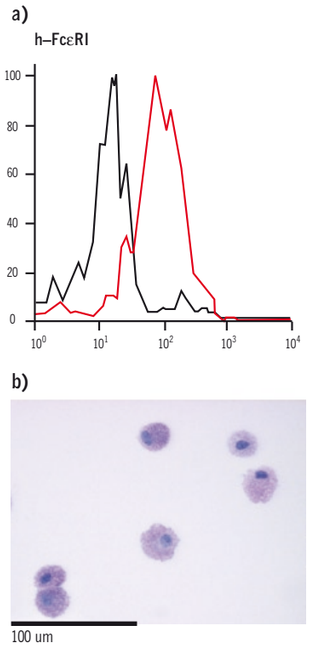
Nicole Rusca and Silvia Monticelli

Adaptive immune responses are vital for the efficient eradication of infectious agents, although deregulated responses might also lead to autoimmune and chronic inflammatory diseases. Some of the key components of adaptive immune responses are $CD4^+$ T helper (Th) lymphocytes. Upon encounter with the antigen, naive $CD4^+$ T cells can differentiate into at least two sub-types of effector cells known as Th1 and Th2, which are characterized by the profile of cytokines they produce (namely, IFN-γ for Th1 cells, and IL-4, IL-5 and IL-13 for Th2 cells). Although $CD4^+$ T cells critically determine the outcome of an infection through the secretion of cytokines, an alteration of the balance between Th1 and Th2-type cytokines might lead to autoimmune diseases, allergy or asthma. The Th1/ Th2 equilibrium must be therefore carefully regulated. MiRNAs are emerging as major players in the regulation of endogenous gene expression in immune homeostasis. The elevated tissue-specific expression of some miRNAs suggests that they might be involved in tissue differentiation and maintenance of cell-type identity. Indeed, the importance of miRNAs in regulating the differentiation and function of cells of the immune system has been demonstrated in mouse models where expression of selected miRNAs (or of the entire miRNA biogenesis pathway) has been altered or eliminated. We are investigating the role of miRNAs in the differentiation of murine $CD4^+$ lymphocytes, in particular from naive T lymphocytes to the Th1 and Th2 subsets. Our preliminary data using miRNA arrays, showed that miR-146a was the only miRNA differentially expressed between these two subsets. We are now studying the role of miR-146a in Th1/ Th2 differentiation as well as its transcriptional regulation in these cells. To this purpose, we are using overexpression and ablation of miR-146a in primary T cells to identify its role during T helper cell differentiation under Th1-, Th2-, or non skewing-conditions. One of the targets for miR-146 is the TNF receptor-associated factor 6 (TRAF6) mRNA. Interestingly, miR-146 expression levels mirror TRAF6 expression during Th1/ Th2 differentiation from naive $CD4^+$ T cells. It has also been shown that miR-146 is part of a negative feedback loop acting on the NF-κB pathway through downregulation of IRAK1 and TRAF6, and we are now investigating whether such molecular circuitry might have a role in fine-tuning T cell differentiation. In fact, although the inflammatory response is essential for dealing with pathogens, if unregulated it can lead to serious disease; thus, understanding the negative regulators of this response is critically important.

Dissecting the molecular mechanisms of human mast cell development and mastocytosis

Lorenzo Dehò and Silvia Monticelli

Human mast cells (hMC) are long-lived cells derived from hematopoietic progenitor cells. They usually don't circulate in mature form in the blood, and instead their differentiation and maturation take place in the tissues where the cells will ultimately reside. HMCs can be found in most tissues, but especially in the ones that are the most exposed to the environment (skin, mucosal airways and gastrointestinal tract), where they contribute to innate bacterial clearance, enhancement of adaptive immune responses, modulation of inflammation and degradation of toxic peptides and venoms. Abnormal accumulation of MCs and/or MC products leads to mastocytosis. However, there are many variants of this disease, presenting a wide range of symptoms and prognosis, and our knowledge on the underlying molecular mechanisms is far from being comprehensive. Since miRNAs are established players in the carcinogenic transformation of several cell types, as well as important regulators of most molecular pathways investigated so far, our goal is to understand the role of miRNAs in the differentiation and effector functions of hMCs, as well as a possible role in mastocytosis development. In fact, to understand what goes wrong when a cell becomes transformed requires also knowledge of the processes that ensure normal development. To answer these questions, we set up in the lab an *in vitro* model of differentiation, where hMCs are differentiated starting from hematopoietic progenitors obtained from peripheral blood and cultured for 6-12 weeks in the presence of a cocktail of cytokines. The resulting cells express the relevant MC markers FcεRI and c-KIT, and show the characteristic metachromatic staining of the cytoplasmic granules. This system will allow us to study the expression of miRNAs in these cells during differentiation and will provide a model in which we will be able to modify miRNA expression and assess the effect on hMC development and functions. Furthermore, we will be able to culture, differentiate and analyze hMCs coming from patients presenting different variants of mastocytosis. Ultimately, we look forward to understanding how perturbation of miRNA-related networks may apply to disease conditions, with the hope that they may lend diagnostic insight or lead to novel entry points for therapeutic intervention.



← *Human mast cells can be differentiated from CD133+ precursors isolated from peripheral blood. a)* *Expression of the typical mast cell markers FcεRI and c-KIT on the surface of cells differentiated in vitro for six weeks (red line; in grey is the isotype control).* ***b)*** *Toluidine blue staining of week-6 mast cells cytospun on a glass slide, showing the characteristic metachromatic purple staining of the cytoplasmic granules.*

Details from July 2007 until June 2009

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Publications

- ***A role for microRNAs in the development of the immune system and in the pathogenesis of cancer.***
  Kanellopoulou C, Monticelli S.
  Semin Cancer Biol. 2008;18:79-88.
- ***Genomics and the immune system.***
  Pipkin M E, Monticelli S.
  Immunology. 2008;124:23-32.
- ***MicroRNA-221-222 regulate the cell cycle in mast cells.***
  Mayoral R J, Pipkin M E, Pachkov M, van Nimwegen E, Rao A, Monticelli S.
  J Immunol. 2009;182:433-445.

Lectures and Seminars

- ***Istituto Clinico Humanitas***
  Seminar "Analysis of microRNA expression in mast cells"
  Milan (IT) / 03.03.2008
- ***Belgian Association for Cancer Research (BACR) Annual Meeting on MicroRNAs and Cancer***
  Bruxelles (BE) / 31.01.2009
- ***Keystone Symposium "MicroRNA and Cancer"***
  Keystone, CO (US) / 10.06.2009

Federica Sallusto
Cellular Immunology

Federica Sallusto

Federica Sallusto received the degree of Doctor in Biology at the University of Rome "La Sapienza" in 1988. 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 staff scientist. She worked at the Basel Institute for Immunology as a visiting scientist in Antonio Lanzavecchia's laboratory from 1993 to 1994 and as a member from 1996 to 2000. Her research is focused on dendritic cell (DC) 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 received the Pharmacia Allergy Research Foundation Award in 1999. Since 2000 she is Head of the Cellular Immunology Laboratory at the IRB.

Research Focus

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 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 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 further investigating the hetherogeneity of memory T cell subsets and the role these subjects play in different physiological and pathological conditions.

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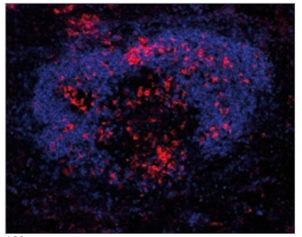
Dissecting the reciprocal interaction between T helper cells and B cells during an immune response in vivo

Dirk Baumjohann and Federica Sallusto

Cognate interaction of B cells with antigen-specific T helper cells in secondary lymphoid organs is required for production of high-affinity antibodies and for generation of memory B cells and long-lived plasma cells. To gain insight into the nature of signals regulating these different aspects of B cell responses, we set up an experimental system in which CD3ε-deficient mice – which lack T cells but have normal B cell development – were reconstituted with small numbers of TCR-transgenic T cells. Serum antibody levels, germinal center reaction, as well as generation of antibody-secreting cells and long-lived plasma cells were monitored at different time points after primary and secondary immunization. We found that adoptively transferred antigen-specific $CD4^+$ T cells induced effective primary antibody responses; however, serum antibody levels were not sustained. Further analysis revealed that upon immunization, T cells proliferated extensively and became exhausted, did not respond to secondary antigenic stimulation, and failed to provide B cell help upon booster immunization. Although immunoglobulin class switch recombination, initiation of germinal center reaction, and differentiation to antibody-secreting short-lived plasma blasts were normal in reconstituted $CD3\varepsilon^{-/-}$ mice, affinity-maturation was impaired and long-lived plasma cells were not generated nor did they migrate to the bone marrow. The results of the study suggest the existence of distinct early and late T cell-dependent events in initiation and maintenance of humoral immune responses.



100 x

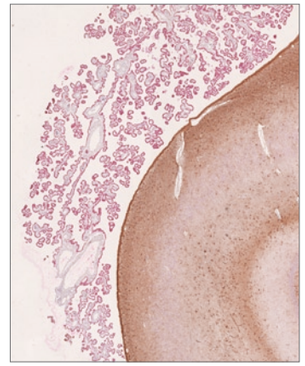
← OT-2 TCR transgenic T cells are primed and migrate to the B cell follicles in $CD3\varepsilon^{-/-}$ mice. *Naive OT-2 cells were adoptively transferred into $CD3\varepsilon^{-/-}$ mice that were then immunized intraperitoneally 16 h later with ovalbumin and MLP. Spleen was dissected on day 10 and fixed cryosections were stained with antibodies to IgD (in blue) to identify B cell follicles and antibodies to CD45.1 (in red) to identify the transferred OT-2 cells.*

CCR6-dependent entry of Th17 cells into the CNS through the choroid plexus initiates EAE

Andrea Reboldi, Dirk Baumjohann and Federica Sallusto

Th17 cells play an important role in EAE but their route of entry into the central nervous system (CNS) and their contribution relative to other effector T cells remain to be determined. Mice lacking CCR6, a chemokine receptor characteristic of Th17 cells, developed Th17 responses but were highly resistant to induction of EAE. Disease susceptibility was reconstituted by transfer of wild-type T cells that entered into the CNS before disease onset and triggered a massive CCR6-independent recruitment of effector T cells across activated parenchymal vessels. The CCR6 ligand CCL20 was constitutively expressed in epithelial cells of choroid plexus in mice and humans. These results reveal distinct molecular requirements and ports of lymphocyte entry into non-inflamed versus inflamed CNS and suggest that the CCR6-CCL20 axis in the choroid plexus controls immune surveillance of the CNS.

» *Reboldi A, et al.* Nat Immunol. 2009; 10:514-523.



➔ CCL20 is expressed by epithelial cells of the choroid plexus. *The image shows a section of human brain tissue in which the choroid plexus epithelial cells are decorated in fuchsia by an antibody to the CCR6 ligand CCL20 and astrocytes are decorated in brown by an antibody to glial fibrillary acidic protein (GFAP).*

On the role of IL-1β in mouse Th17 differentiation and Th17-mediated immunopathology

Francesca Ronchi, Andrea Reboldi and Federica Sallusto

Th17 cells represent a recently identified subset of T helper (Th) cells characterized by the production of IL-17 and IL-22 and whose differentiation is under the control of the transcription factors RORγt and RORα. IL-17 has been shown to mediate protection against extracellular pathogens by promoting neutrophil recruitment but also to cause immunopathology in different models of autoimmunity, including experimental autoimmune encephalomyelitis (EAE). In mice differentiation of Th17 cells from naive T cell precursors is dependent on IL-6 and TGF-β whereas a combination of IL-1β and IL-6 has been shown to be important for human Th17 differentiation. Mice deficient for Caspase-1, a cysteine protease that induces proteolytic cleavage and export of proinflammatory cytokines such as IL-1β, IL-18 and IL-33, are resistant to induction of EAE. However whether this defect coincides with a defect in generation or function of Th17 cells has not been addressed. In a first series of *in vitro* experiments we primed naive T cells with dendritic cells and CD3 antibodies under standard Th1-, Th2- and Th17-polazing conditions. We found that naive T cells from Caspase-1-KO mice were able to differentiate into Th1, Th2 and Th17 effector cells when primed *in vitro* with either Caspase-1-KO or wild type DC. Similarly, wild-type naive T cells differentiated to polarized effector cells when primed by Caspase-1-KO dendritic cells. We then assessed T cell differentiation *in vivo*. Caspase-1-KO and wild-type mice were immunized with soluble antigen (Ag) admixed with different adjuvants. Capsase--KO and wild type mice showed comparable Th1, Th2 or Th17 response. Thus, impaired T cell priming or differentiation is not sufficient to explain the resistance to EAE induction observed in Caspase-1-KO mice.

IL-22 but not IL-17 production by a subset of human skin-homing memory T cells

Thomas Duhen, Rebekka Geiger and Federica Sallusto

IL-22 is a cytokine produced by Th17 and NK cells which acts on epithelial cells and keratinocytes and has been implicated in skin homeostasis and inflammation. We characterized a population of human skin-homing memory CD4$^+$ T cells that expresses CCR10, CCR6 and CCR4 and produces IL-22 but not IL-17 nor IFN-γ. Clones isolated from this population produced IL-22 only and expressed low or undetectable amounts of the Th17 and Th1 transcription factors RORγt and T-bet. Differentiation of T cells producing IL-22 only was efficiently induced in naive T cells by plasmacytoid dendritic cells in an IL-6- and TNF-dependent fashion. These findings delineate a novel subset of human CD4$^+$ effector T cells dedicated to skin physiology.

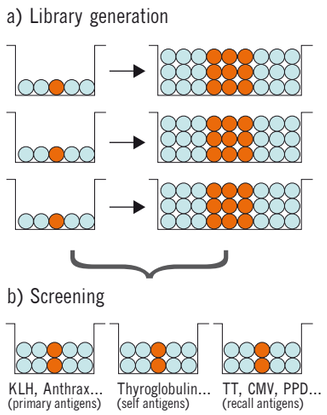
» ***Duhen T, et al.***
Nat Immunol. 2009;
10:857-863.

Human naive and memory CD4$^+$ T cell repertoires specific for naturally processed antigens analyzed using libraries of amplified T cells

Rebekka Geiger, Thomas Duhen and Federica Sallusto

The enormous diversity of the human naive CD4$^+$ T cell repertoire is instrumental to the generation of an immune response to virtually any foreign antigen that can be processed into peptides which bind to MHC class II molecules. The low frequency of antigen-specific naive T cells, their high activation threshold and the constrains of antigen-processing and presentation have hampered the analysis of the naive repertoire to complex protein antigens. In this study libraries of polyclonally expanded naive T cells were used to determine frequency, epitope specificity and functional avidity of human naive CD4$^+$ T cells specific for a variety of antigens and to isolate antigen-specific T cell clones. In the naive repertoire, T cells specific for primary antigens, such as KLH and Anthrax protective antigen, and for recall antigens, such as tetanus toxoid, cytomegalovirus and *Mycrobacterium tuberculosis*, were detected at frequencies ranging from 5 to 170 cells per 10^6 naive CD4$^+$ T cells. These cells showed a broad range of functional avidities over 3 orders of magnitude. In contrast, in the memory repertoire T cells specific for primary antigens were not detected, while T cells specific for recall antigens were detected at high frequencies and displayed homogenously high functional avidity. The method described may find applications for rapid identification of antigenic sites in vaccine candidates, for testing antigenicity of therapeutic proteins, drugs and chemicals, and for generation of antigen-specific T cell clones for adoptive cellular immunotherapy.

» ***Geiger R, et al.***
J Exp Med. 2009;
206:1525-1534.



← *Schematic representation of the T cell library method*. *a)* *Library generation: naive CD4$^+$ T cells are seeded at 2,000 cells/well in multiple wells containing irradiated allogeneic PBMC, PHA and IL-2. The individual cultures are expanded into larger wells that make up the library of amplified T cell blasts.* ***b)*** *Screening of the library: T cells from individual cultures are collected, washed and tested for their capacity to proliferate in response to various antigens in the presence of autologous antigen-presenting cells.*

Prostaglandin E2 enhances Th17 responses by differentially modulating the release of IL-17 and IFN-γ by purified memory CD4$^+$ T cells

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

The contribution of Th1 and Th17 cells in chronic inflammatory conditions leading to autoimmunity remains highly controversial. Prostaglandins have been shown to favor Th17 responses indirectly by increasing IL-23 and blocking IL-12 release from antigen presenting cells. We found that PGE2 can directly modulate cytokine production by human memory T cells. TCR triggering in the presence of PGE2 increased IL-17 and reduced IFN-γ production by freshly isolated memory T cells or T cell clones. PGE2 triggered the EP2 and EP4 receptors expressed on T cells leading to a rapid increase of ROR-γt and decrease of T-bet mRNA. Moreover, PGE2 promoted the selective enrichment of IL-17 producing cells *in vitro* by differentially modulating the proliferation of memory T cell subsets. Taken together our results indicate that T cell effector function is a direct target for PGE2 modulation and suggest a novel mechanism to elucidate the anti-inflammatory effect of COX-2 inhibitors.

» ***Napolitani G, et al.***
Eur J Immunol.
2009;
39:1301-1312.

CD40L$^+$ CD4$^+$ effector memory T cells migrate in a CD62P-dependent fashion into reactive lymph nodes and license dendritic cells for T cell priming

Alfonso Martín-Fontecha, Dirk Baumjohann, Greta Guarda, Andrea Reboldi and Federica Sallusto

There is growing evidence that the maturation state of dendritic cells (DCs) is a critical parameter determining the balance between tolerance and immunity. We report here that mouse CD4$^+$ effector memory T cells (T_{EM}), but not naive or central memory T cells (T_{CM}), constitutively expressed CD40L at levels sufficient to induce DC maturation *in vitro* and *in vivo* in the absence of antigenic stimulation. CD4$^+$ T_{EM} cells were excluded from resting lymph nodes but migrated in a CD62P-dependent fashion into reactive lymph nodes that were induced to express, in a transient or sustained fashion, CD62P on high endothelial venules. Trafficking of CD4$^+$ T_{EM} cells into chronic reactive lymph nodes maintained resident DCs in a mature state and promoted naive T cell responses and experimental autoimmune encephalomyelitis (EAE) to antigens administered in the absence of adjuvants. Antibodies to CD62P, that blocked CD4$^+$ T_{EM} migration into reactive lymph nodes, inhibited DC maturation, T cell priming and induction of EAE. These results show that T_{EM} cells can behave as endogenous adjuvants and suggest a mechanistic link between lymphocyte traffic in lymph nodes and induction of autoimmunity.

» ***Martín-Fontecha A, et al.***
J Exp Med. 2008;
205:2561-2574.

Analysis of the human antibody response to Dengue virus and production of a broadly neutralizing cocktail of human monoclonal antibodies lacking infection enhancing activity

Martina Beltramello, Antonio Lanzavecchia and Federica Sallusto

Dengue virus (DENV) infection is a large and growing public health problem in Asia and the Americas. Antibodies are postulated to be central to both DENV immunity and pathogenesis. In particular, antibody-dependent enhancement (ADE) is suggested to explain the association between heterotypic secondary infection and severe disease. To understand better antibody mediated neutralization and enhancement, we immortalized memory B cells from individuals recovered from primary or secondary DENV infection and isolated a large panel of human monoclonal antibodies (mAbs) specific for both DENV structural and non-structural proteins. The human response was dominated by antibodies to domain I and II of the surface E protein and to prM. These antibodies were broadly cross-reactive with the four DENV serotypes and neutralized weakly or failed to neutralize infection of susceptible targets, while potently enhancing infection of cells expressing Fc-γ receptors. In contrast, the few domain III-specific antibodies isolated showed potent neutralizing activity as well as modest enhancing activity. To develop an antibody cocktail capable of neutralizing, but not enhancing infection by all DENV serotypes, we selected three human mAbs and expressed them recombinantly with two mutations that prevent binding to FcγR. Together, these recombinant antibodies target two epitopes of the E protein in each serotype, thus reducing the risk of emergence of escape mutants. When tested *in vitro* this human antibody cocktail retained full neutralizing activity but failed to enhance infection.

Self-limitation of human effector Th17 cell responses by reciprocal regulation of IL-10 and IL-17

Christina Zielinski and Federica Sallusto

Th17 cells have recently emerged as a new T cell lineage that trigger production of pro-inflammatory cytokines and chemokines by a broad range of cellular targets. Although these effector functions confer Th17 cells the ability to protect against certain extracellular bacteria and fungi, an uncontrolled Th17 response can induce severe tissue destruction and autoimmunity. Therefore, mechanisms must be in place to shield the host from immune-mediated damage. The aim of this study is to investigate the stability and flexibility of the pro-inflammatory human Th17 subset and to identify factors that can modulate the cytokine profile of differentiated Th17 cells. We found that Th17 cells can produce immunosuppressive cytokine IL-10. IL-10 can be induced in the $CCR6^{+}CCR4^{+}$ Th17 cell subset, as well as in Th17 clones, under certain stimulatory conditions and is accompanied by down-regulation of IL-17. We also found that the Th17 phenotype is subject to a complex modulation by other cytokines. IL-23, for example, promoted the regulatory Th17 phenotype by up-regulation and maintenance of IL-10/IL-17 co-expression. This reciprocal regulation of pro- and anti-inflammatory cytokines might constitute a self-regulatory mechanism that allows for self-limitation of the inflammatory Th17 cell response.

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Funding

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  Memory T cell subsets in man and mouse
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- ***Bill & Melinda Gates Foundation***
  PTVDC: Poxvirus T cell vaccine discovery consortium
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- *European Union*
  MAIN: Targeting cell migration in chronic inflammation
  FP6 - LSHG-CT-2003-502935 / 2003-2008
- *European Union*
  DC-THERA: Dendritic cells for novel immunotherapies
  FP6 - LSHB-CT-2004-512074 / 2005-2010
- *European Union*
  INNOCHEM: Innovative chemokine-based therapeutic strategies for autoimmunity and chronic inflammation
  FP6 – LSHP-CT-2005-518167 / 2005-2010
- *European Union*
  SENS-IT-IV: Novel testing strategies for in vitro assessment of allergens
  FP6 - LSHB-CT-2006-01868 / 2005-2010
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  2008-2009
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  Isolation of human monoclonal antibodies to understand Dengue virs neutralization and enhancement
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  20386 / 2006 - 2010

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Publications

- ***Division of labor with a workforce of one: challenges in specifying effector and memory T cell fate.***
  Reiner S L, Sallusto F, 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.***
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  Eur J Immunol. 2008;38:30-39.
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  Eur J Immunol. 2008;38:2076-2084.
- *Chemokines and leukocyte traffic.*
  Sallusto F, Baggiolini M.
  Nat Immunol. 2008;9:949-952.
- *CD40L+ CD4+ memory T cells migrate in a CD62P-dependent fashion into reactive lymph nodes and license dendritic cells for T cell priming.*
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Book chapters

Martin-Fontecha A, Lanzavecchia A, Sallusto F."Dendritic cell migration to peripheral lymph nodes". In Handbook of Experimental Pharmacology. Lombardi G and Riffo-Vacquez Y Eds. Springer, Berlin Heidelberg. 2009

Lectures and Seminars

- *11th Annual Meeting of SFCI*
  Tokyo (JP) / 11.07.2007
- *Annual Congress of the "Deutschen, Oesterreichischen und Schweizerischen Gesellschaft für Hämatologie und Onkologie"*
  Basel (CH) / 05.10.2007
- *XLIV National Congress of the Italian society of Rhemutalogy*
  Venezia Lido (IT) / 17.10.2007
- *Annual Meeting of the Croatian Immunological Society*
  Rovinj (HR) / 19.10.2007
- *15th Annual Meeting of the International Cytokine Society "Cytokines in Health and Disease"*
  San Francisco (US) / 26.10.2007
- *University of Freiburg, Symposium "A day of immunology in Freiburg"*
  Freiburg (DE) / 30.11.2007
- *37th Annual Scientific Meeting of the Australasian Society for Immunology*
  Sydney (AU) / 02.12.2007
- *MASIR 2008*
  La Plagne (FR) / 30.01.2008
- *Ringberg Colloquium on "Determinism and plasticity of T lymphocytes"*
  Rottach-Egern (DE) / 10.02.2008
- *Berzelius Symposium*
  "Vaccine development in the 21st century"
  Stockholm (SE) / 19.02.2008
- *Symposium Immunotherapy SFB 685*
  Tuebingen (DE) / 06.03.2008
- *BioSymposia "Tregs and Th17 Cells in Autoimmunity"*
  Washington (US) / 25.03.2008
- *FOCIS 2008*
  Boston (US) / 05.06.2008
- *VI National congress of the Italian Society for Clinical Immunology and Allergology*
  Rome (IT) / 11.06.2008
- *European League against Rheumatism (EULAR) Annual Meeting*
  Paris (FR) / 11.06.2008
- *London Immunology Group "Leukocyte differentiation and regulation in disease"*
  London (UK) / 12.09.2008
- *22nd European Machrophage & Dendritic Cell Society Annual Meeting*
  Brescia (IT) / 18.09.2008
- *Gordon Research Conference "Chemotactic Cytokines"*
  Aussois (FR) / 21.09.2008
- *The 10th International Symposium on Dendritic Cells*
  Kobe (JP) / 01.10.2008
- *The American College of Rheumatology, Annual Meeting*
  San Francisco (US) / 24.10.2008
- *Centre d'Immunologie de Marseille-Luminy*
  Keynote seminar"Lymphocyte trafficking in immunity and autoimmunity"
  Marseille (FR) / 13.11.2008
- *International Congress on Cytokines in Immune Regulation and Disease*
  Florence (IT) / 04.12.2008
- *Keystone Symposium "Mobilizing Cellular Immunity for Cancer Therapy"*
  Snowbird (US) / 11.01.2009
- *Harvard Medical School*
  Immunology Seminar Series "Lymphocyte trafficking in immunity and autoimmunity"
  Boston (US) / 04.02.2009
- *Massachusetts General Hospital*
  Immunology Seminar Series "Human memory T cells: heterogeneity, stability and flexibility"
  Boston (US) / 05.02.2009
- *Keystone Symposium "TH17 Cells in Health and Disease"*
  Vancouver (CA) / 05.02.2009
- *Keystone Symposium "Immunologic Memory and Host Defense"*
  Keystone, CO (US) / 08.02.2009
- *Accademia Medica di Rome*
  Lecture"Migrazione e funzione effettrice dei linfociti T nelle patologie autoimmuni"
  Rome (IT) / 05.03.2009
- *World Immune Regulation Meeting-III*
  Davos (CH) / 21.03.2009
- *University of Genoa*
  Seminar"Lymphocyte trafficking in immunity and autoimmunity"
  Genoa (IT) / 22.05.2009

- *University of Edinburgh*
  The Edinburgh Immunology Group Seminar series"Th17 cell trafficking in immunity and autoimmunity"
  Edinburgh (UK) / 28.05.2009
- *8th Elsinore Meeting on Infection Immunity "Prophylaxis and therapeutic intervention in host-yethogen interaction"*
  Helingoer (DK) / 05.06.2009
- *3rd Tagernsee Conference "Immunotherapy of Cancer"*
  Keynote Lecture
  Lake Starnberg (DE) / 02.07.2009

Marcus Thelen
Signal Transduction

Marcus Thelen

Marcus Thelen studied biochemistry at the University of Tuebingen (DE). He received his PhD from the University of Bern. Then, he moved to the Thodor-Kocher-Institute in Bern where his interest started to focus on inflammation and chemokines. In 1989 he went to the Rockefeller University in New York joining the group of Alan Aderem in the Laboratory of Cellular Physiology and Immunology of Cohn/Steinman department. Biochemical aspects of cyotokine- and endotoxin-mediated phagocyte priming and cytoskeleton-mediated signal transduction were the topics of his studies then. In 1992 he was received a career development award (START) from the Swiss National Science Foundation and returned to the Theodor-Kocher-Institute at the University of Bern. He created his own research group working on molecular mechanisms of signal transduction in leukocytes focusing on PI3-kinase-dependent pathways and chemokine-mediated receptor activation. In 1994 he obtained the *venia docendi* from the University of Bern and was later in 2001 awarded an honorary professorship from the same University. In 2000 he moved to Bellinzona and assisted in the opening of the IRB. Marcus Thelen heads since then the Laboratory of Signal Transduction.

Research Focus

The chemokine system is best known for its role in the regulation of leukocyte trafficking. Chemokine receptor-induced signal transduction in leukocytes is essential for leukocyte homing to lymphoid organs and for recruitment of immune cell to sites of inflammation, but in addition the chemokine system mediates migration during embryogenesis, angiogenesis and tumor cell spreading. Common to all is that the cells migrate along a guidance cue formed by a gradient of an appropriate chemokine. Chemokines are produced by various cell types, *e.g.* endothelium, epithelium and stromal cells. Recent studies revealed an important additional function of the chemokine system. So called silent receptors which do not promote typical signaling, can scavenge selective chemokines thereby contributing to the formation of gradients. CXCR7/RDC1, a recently deorphanized receptor for CXCL12, appears to possess such activity. Downstream of the receptors small GTPases of the Rho family modulate the actin cytoskeleton. The GTPases become activated by different guanine exchange factors (GEFs) depending on the cellular context. The group contributes to the elucidation of the molecular mechanisms which regulate cell migration in leukocytes and tumor cells. Next to molecular biology and biochemical methods, high-end confocal microscopy is employed to visualize protein activities and to characterize their distribution in migrating cells.

Team

Group Leader: Marcus Thelen, PhD › *marcus.thelen@irb.unisi.ch*

Members: Tiziana Apuzzo, *PhD student* – Elisabetta Cameroni, *PhD* – Ulrike Naumann, *PhD student* – Sylvia Thelen, *PhD* – Silvia Volpe, *PhD student*

Novel CXCR4-activated signal transduction pathways

Tiziana Apuzzo and Marcus Thelen

Chemokine receptors belong to the rhodopsin-like subfamily of heptahelical G-protein coupled receptors and share the ability to mediate leukocyte migration. The ubiquitously expressed chemokine receptor CXCR4 was shown to have in addition to its function on leukocytes a critical role during embryogenesis and hematopoieis. The marked appearance on many invasive cancer tissues correlates with the ability of CXCR4 to mediate tumor cell migration. Cancer cell migration differs from the amoeboid motility of leukocytes and resembles more the mesenchymal mode of migration used by fibroblast and smooth muscle cells. Mesenchymal migration is characterized by a much slower movement than observed with leukocyte and depends on stronger adhesion sites. CXCR4 further distinguishes from other chemokine receptors through its signaling properties and functional responsiveness. Like other chemokine receptors CXCR4 is coupled to pertussis toxin sensitive heterotrimeric G_i-proteins, but has the ability to trigger the prolonged activation of intracellular signal transduction. Nevertheless, the presence of the receptor on a cell surface does not correlate with its activity profile. Thus, it is conceivable that CXCR4 in addition to G_i-coupling interacts with additional proteins which regulate the characteristic responses of the receptor. The aim of the project is to identify novel interaction partners of CXCR4, which mediate the receptor-typical responses. Several laboratories used the C-terminus of a GPCR as bait in GST-pull down or yeast two hybrid experiments to identify associated proteins. However, the cytoplasmic surface of a chemokine receptor consists not only of the C-terminus, but includes three intracellular loops which connect the transmembrane helices. These loops together with the C-terminus form a large structured surface to which proteins can bind. We developed a protocol which allows the immunoprecipitation of CXCR4 under mild detergent conditions which preserves the native conformation of the receptors and the association with downstream effectors. We have used mass-spectrometry to unveil several so far undisclosed interactions of CXCR4 with cytosolic proteins. Among the proteins we identified potential scaffolding molecules and proteins which could stand for a direct link of CXCR4 with NFκB-dependent pathways and protein *de novo* synthesis. Characterization of the functional role of these interactions is the main focus of our current investigations. In particular we are interested in pathways which could mediate tumor cell migration.

Generation of fluorescent protein tagged chemokines for live cell imaging

Elisabetta Cameroni and Marcus Thelen

Chemokine receptors often bind multiple chemokines and conversely, a chemokine can bind to different chemokine receptors. This well known promiscuity co-regulates the fine tuning of leukocyte trafficking during homing and the recruitment to specific sites of inflammation.

Functional imaging of chemokine receptor interactions in real-time allows the assessment of their relevance in the migratory process. The aim of the recently started project is to generate fluorescent chemokine analogues and derivates which can be used to follow receptor activity in primary tissues. Upon ligand binding chemokine receptors rapidly internalize carrying the bound chemokine as cargo to endosomes. Later, following acidification the low pH of endosomes leads to the dissociation of the receptor and the chemokine. Nevertheless, due to the rapid fusion of early endosomes and the accumulation of the cargo receptor-mediated uptake of fluorescent chemokines can be easily observed by microscopy. Degradation and fading of fluorescence can be attenuated in the presence of acidification blockers, which prevent the lowering of the pH and the activation of lysosomal proteases, but is from our experience not mandatory. Chemokines are small 8-10 kD proteins that can be produced *in vitro* through chemical synthesis or with recombinant expression systems. Both methods have their advantages. We tested several expression systems that should provide access to native and mutated chemokines tagged with different fluorescent proteins. Correct folding and a defined homogeneous N-terminus of the chemokines fused with fluorescent proteins are most critical. We have taken advantage of an arthropod cell based expression system that produces chemokines in the absence of inflammatory mediators, such as lipopolysaccharide (LPS). Chemokines tagged with blue, green, yellow, orange or red fluorescent proteins stimulate leukocyte migration as efficiently as their wild type counterparts. Incubation of fresh tissue sections reveal locally restricted selective uptake of chemokines. Studies on fresh tissue specimens are in progress and should provide new insight on regulated receptor activity. Furthermore application of the fluorescent tagged chemokines *in vitro* will be used to explore chemokine receptor activity in cells cultured under different condition.

G-protein coupled receptor activity in migrating cells

Silvia Volpe and Marcus Thelen

Cell migration is a well described phenomenon which is critical during development, immune homeostasis and responses as well as for tissue repair. In general, cells migrate along a guidance cue which is formed by a chemotactic gradient. The relative concentration difference of a chemoattractant between the front and the rear of a motile cell within such gradient is minute (< 10%). Nevertheless, cells can easily sense the gradient and polarize forming a characteristic leading edge and a trailing uropod. Intracellular polarization is realized by locally activated enzymes, *e.g.* PI 3-kinase activity is stimulated in the front whereas pathways dependent on the small GTPase Rho are activated at the rear. Leukocytes rapidly assume the typical morphology when moving in a shallow gradient of chemoattractants. The small concentration difference of the chemoattractant along the polarization axis is probably insufficient to stimulate different receptor efficiencies at the front and the rear. To explain the functional morphology, several mechanisms for cell polarization have been

» ***Thelen M, Stein JV*** *Nat Immunol.* 2008; 9:953-959.

proposed an asymmetric receptor distribution, local differences in receptor occupancy, or intracellular feed-back signaling mechanisms. Most chemotactic movement in mammalian cells is stimulated via G_i-coupled seven transmembrane containing receptors (GPCRs). Recently receptors tagged with fluorescent probes were described to observe the activation of GPCRs by fluorescence energy transfer (FRET). We use alpha adrenergic receptors tagged with CFP and YFP to measure their spatio-temporal activation in a chemotactic gradient by time-lapse video microscopy. Confocal section analyses reveals an equal distribution of chemoattractant receptors over the entire plasma membrane in cells moving along a chemotactic gradient. We can time resolve the activation of selective intracellular pathways at the leading edge, suggesting a non-uniform signaling downstream of chemoattractant receptors. Reversion of the chemotactic gradient appears not to affect the overall receptor activity at the plasma membrane, but relocalizes downstream signaling cascades. The observations are consistent with a subsecond signaling of the receptors leading to the suppression of the activation of downstream signal transduction pathways.

Functional characterization of CXCR7 in mammalian tissues

Ulrike Naumann and Marcus Thelen

Previous phylogenetic analyzes indicated that RDC1 is a G-protein coupled receptor with similarity to chemokine receptors. We recently unveiled that RDC1 acts as second receptor for the chemokine CXCL12 (SDF-1), the receptor was therefore renamed to CXCR7. Although the receptor binds CXCL12 and CXCL11/ITAC with high affinity, characteristic chemokine signaling could not be demonstrated and the receptor has not yet found general consent as typical chemokine receptor. In contrast to other chemokine receptors CXCR7 does not stimulate chemotaxis. CXCR7 is widely expressed on leukocytes and like CXCR4 on many somatic cells, including tumors. Several studies have linked the expression of CXCR7 with tumor aggressiveness and spreading. However, a functional activity of CXCR7 for tumor growth has not yet been identified. On leukocytes CXCR7 is most prominently expressed on monocytes and B cells. On the latter the expression is tightly regulated during development, being most prominent on circulating $CD19^+$ B cells. On mature $CD19/CD27^+$ memory B cells CXCR7 expression correlates with the ability of the cells to differentiate to antibody producing plasma cells. Recent observations in zebra fish suggest that the receptor acts a scavenger for CXCL12. Somatic cells take up CXCL12 thereby generating a gradient for the migration of primordial germ cells. The current project aims at the characterization of a similar activity for the mammalian receptor. Indeed, we found that primary human endothelial cells display scavenging activity for CXCL12 which can be attributed to CXCR7. A second focus of the project aims in resolving a potential coupling to G-proteins. The lack of typical G_i-protein-mediated signaling poses the question if the receptor associates with another subclass of G-proteins or if the receptor does not signal through any G-protein. Transposition where domains of CXCR7 and of the related CXCR4 will be exchanged should give insight into the coupling and signal transduction mechanisms of CXCR7.

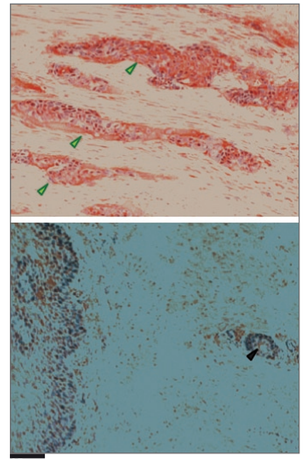
» ***Thelen M, Thelen S***
J Neuroimmunol. 2008; 198:9-13.

The guanine nucleotide exchange factor for Rac P-Rex1

Sylvia Thelen and Marcus Thelen

The small GTPases of the Rho family Rac and Cdc42 are critical for rapid rearrangements of the actin cytoskeleton observed during filopodia and lamellipodia formation in migrating cells. The GTPases act as switches and are either 'on' in their GTP bound form or 'off' when loaded with GDP. Activation of the GTPases is catalyzed by specific GTP exchange factors (GEF). The phosphatidylinositol 3,4,5-trisphosphate (PIP_3)-dependent exchanger 1 (P-Rex1) is assumed to be involved in G-protein coupled receptor (GPCR) mediated Rac activation. P-Rex1 activity is stimulated by the PI 3-kinase product PIP_3 and by the βγ–subunits of heterotrimeric G-proteins, which are released upon activation of GPCRs. Consistent with the activation by these cofactors and their cellular localization, following stimulation of the cells P-Rex1 is recruited to the plasma membrane. Over expression of P-Rex1 or its suppression by siRNA markedly alters chemokine-stimulates migratory capacity of myeloid leukocytes, consistent with the assumption the GEF is required for efficient chemotaxis. P-Rex1 becomes phosphorylated at multiple sites following cell activation and the modification appears to contribute to its subcellular localization. The project aims on the molecular characterization of different domains P-Rex1 and their role in chemokine receptor-mediated signal transduction. To further investigate the expression of P-Rex1 in different tissues and to better reveal its subcellular localization we generated monoclonal antibodies suitable for immunofluorescence analysis and immunohistochemistry. Staining of normal epithelium and invasive cancer from human esophagus reveals a marked up-regulated expression of P-Rex1 in the tumor. The data suggest a potential role of P-Rex1 for tumor cell migration and invasion.

» ***Barber MA, et al.***
J Biol Chem. 2007; 282:29967-29976.



➔ ***P-Rex1 expression in human esophagus epithelium.*** **Upper panel:** *normal tissue. P-Rex1 is scarcely expressed in the epithelium. The arrowhead points to the endothelium of a blood vessel which is P-Rex1 positive.* **Lower panel:** *Invasive carcinoma. P-Rex1 is massively upregulated in the invasive cells.*

Details from July 2007 until June 2009

Funding

- *Swiss National Science Foundation*
  Chemokine receptors and signal transduction pathways in leukocytes
  3100A0-1122141 / 2006-2009
- *San Salvatore Foundation*
  Analysis of chemokine receptor CXCR4 interacting proteins in different tissues
  2006-2009
- *European Union*
  INNOCHEM: innovative chemokine-based therapeutic strategies for autoimmunity and chronic inflammation
  FP6 – LSHP-CT-2005-518167 / 2005-2010
- *European Union*
  MAIN: targeting cell migration in chronic inflammation
  FP6-NoE LSHG-CT-2003-502935 / 2003-2008
- *European Union, Marie Curie Actions*
  INTEGRAMM: International Graduate Program in Molecular Medicine
  20386 / 2006-2009
- *Roche Research Foundation*
  Interactions and subcellular distribution of the potentially tumorigenic chemokine receptor CXCR7/RDC1 in lymphocytes
  223-2007 / 2007-2008
- *Novartis Foundation*
  Interactions and subcellular distribution of the potentially tumorigenic chemokine receptor CXCR7/RDC1 in lymphocytes
  2007-2008
- *Ticino Foundation for Cancer Research*
  Detailed study of the interactions and subcellular distribution of thetumorigenic chemokine receptor CXCR7/RDC1 in lymphocytes
  2009

Collaborations

- *Peter Gierschick*
  University of Ulm (DE)
- *John Hartwig*
  Brigham and Woman Hospital, Havard Medical School, Boston (US)
- *Fernado Arenzana-Seisdedos*
  Pasteur Institute, Paris (FR)
- *Heidi Welch*
  Babarham Institute, Babraham (UK)

Publications

- ***Membrane translocation of P-Rex1 is mediated by G protein betagamma subunits and phosphoinositide 3-kinase.***
  Barber M A, Donald S, Thelen S, Anderson K E, Thelen M, Welch H C.
  J Biol Chem. 2007;282:29967-29976.
- ***CXCR7, CXCR4 and CXCL12: an eccentric trio?***
  Thelen M, Thelen S.
  J Neuroimmunol. 2008;198:9-13.
- ***How chemokines invite leukocytes to dance.***
  Thelen M, Stein J V.
  Nat Immunol. 2008;9:953-959.
- ***A crosstalk between intracellular CXCR7 and CXCR4 involved in rapid CXCL12-triggered integrin activation but not in chemokine-triggered motility of human T lymphocytes and CD34+ cells.***
  Hartmann T N, Grabovsky V, Pasvolsky R, Shulman Z, Buss E C, Spiegel A, Nagler A, Lapidot T, Thelen M, Alon R.
  J Leukoc Biol. 2008;84:1130-1140.

Lectures and Seminars

- *University of Brescia*
  Seminar "RDC1/CXCR7 a novel potential chemokine receptor"
  Brescia (IT) / 04.09.2007
- *Meeting on Hormones and Cell Reguation - GPCR complexes and GPCR complexity*
  Mont St. Odile (FR) / 16.09.2007
- *University of Nottingham*
  Seminar "Intelligent Networks in Intracellular Communication"
  Nottingham (UK) / 11.10.2007
- *Meeting on Chemokines and Chemokine Receptors in the Nervous System*
  Rome (IT) / 28.10.2007

- *University Hospital Zurich Balgrist*
  Seminar "CXCR7 - CXCR4 - CXCL12 a trio with assigned relations"
  Zurich (CH) / 06.05.2008
- *University Hospital Hamburg*
  Seminar "RDC1/CXCR7 a novel potential chemokine receptor"
  Hamburg (DE) / 04.09.2008
- *INNOCHEM Workshop*
  Madrid (ES) / 24.09.2008
- *Leibniz-Institut for Molecular Pharmacology*
  Seminar "Chemokine receptor signaling and scavenging"
  Berlin (DE) / 16.12.2008
- *Institut Pasteur*
  Seminar "Chemokine receptor signaling and scavenging activities for leukocyte migration"
  Paris (FR) / 22.04.2009
- *Pepscan*
  Seminar "Chemokine receptor signaling and scavenging activities for leukocyte migration"
  Lelystad (NL) / 26.05. 2009
- *University of Birmingham*
  Seminar "The Yin yang of chemokine receptor functions"
  Birmingham (UK) / 15.06. 2009
- *University of Cardiff*
  Seminar "Chemokine receptor signaling and scavenging activities during leukocyte migration"
  Cardiff (UK) / 25.06.2009
- *Babrham Institute*
  Seminar "Functional chemokine receptor response"
  Babrham, Cambridge (UK) / 30.06. 2009
- *IBIOS Inaugural Conference on Functional Optical Imaging, University of Nottingham*
  Nottingham (UK) / 03.07. 2009

Mariagrazia Uguccioni
Chemokines in Immunity

Mariagrazia Uguccioni

Mariagrazia Uguccioni received a degree in Medicine from the University of Bologna (IT) where she specialized in Haematology in 1994. From 1993 to 2000 she was a member of the Theodor Kocher Institute, University of Bern (CH), and since 2000 she is group leader at the IRB. She is adjunct professor of Immunology at the School of Rheumatology, University of Bologna (IT), since 2000. Mariagrazia Uguccioni's research has covered aspects of human haematology and Immunology: chemokine expression and activities in normal and pathological conditions, leukocyte activation and traffic, natural chemokine antagonists and synergy-inducing chemokines. Recently, her group is focusing on chemokine activities in human autoimmune diseases, tumors, and infections and has identified a novel regulatory mechanism of leukocyte trafficking induced by synergy-inducing chemokines.

Research Focus

Our research interest remains focused on chemokine activities in physiology and pathology, with emphasis on the mechanisms governing fine tuning modulation of their expression and activity. Research in the chemokine field has dramatically changed our understanding of leukocyte traffic in immune defence and disease. 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, autoimmunity, and tumors.

Structure/function studies. Chemokine structure/function studies led us to identify, on the one hand, chemokines that can act as natural antagonists by preventing natural agonist binding, and the subsequent activation of the receptor. On the other hand, some chemokines that can act in synergism with chemokine receptor agonists. However, the molecular basis of synergy-inducing chemokine activities remains, at present, obscure and the analysis of this phenomenon is part of our ongoing research.

Chronic inflammatory disease and autoimmunity. Our long lasting collaboration with the group of Costantino Pitzalis in London, has led to define a new functional property of synovial helper T cells, demonstrating their active involvement in the local production of B cell chemoattractants, and suggesting their direct contribution in the mechanisms of B cell localization and ectopic lymphoid neogenesis in rheumatoid arthritis.

Inflammation and tumor. Chronic and persistent inflammation contributes to cancer development and even predisposes to carcinogenesis. Infection-driven inflammations are involved in the pathogenesis of approximately 20% of human tumors. Moreover, even tumors which are not epidemiologically linked to pathogens are characterized by the presence of an inflammatory component in the tumor microenvironment. Human cancers possess a complex chemokine network that influences the extent and phenotype of the leukocyte infiltrate, as well as tumor cell and endothelial cell growth and migration. We are therefore expanding our studies on the tumor microenvironment in extranodal lymphomas, focusing on the expression of chemokines that could account for both lymphocyte infiltration, and positioning of malignant B cells in the perivascular cuff.
Chemokines in HIV/SIV infection. Chemokine expression changes, that different vaccination strategies can induce in secondary lymphoid organs and at mucosal sites, might account for the different adaptive immune responses and could be used to identify efficient vaccines. We are pursuing these studies in the frame of projects funded by the European Union.

Team

Group Leader: Mariagrazia Uguccioni, MD > *mariagrazia.uguccioni@irb.unisi.ch*
Members: Denise Bottinelli, *PhD student* – Valentina Cecchinato, *PhD* – Maria Gabriela Danelon, *Technician* – Katrin Kuscher, *PhD student* – Milena Schiraldi, *PhD student* – Luisa Stefano, *PhD* – Daniel Venetz, *PhD student*

Chemokines: structure/function studies

Katrin Kuscher, Milena Schiraldi, Luisa Stefano and Mariagrazia Uguccioni

The migration of monocytes to sites of inflammation is largely determined by their response to chemokines. While the chemokine specificities and expression patterns of chemokine receptors are well defined, it is still a matter of debate how cells integrate the messages provided by different chemokines that are concomitantly produced in physiological or pathological situations *in vivo*. 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 have studied 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 cellular responses. The data 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.

» ***Kuscher K, et al.*** Eur J Immunol. 2009; 39:1118-1128

Chemokines in chronic inflammation and autoimmunity

Denise Bottinelli, Milena Schiraldi and Mariagrazia Uguccioni

The chemokine system offers many potential entry points for innovative anti-inflammatory therapies in chronic inflammation, as well as autoimmune diseases, such as Multiple Sclerosis, Rheumatoid Arthritis (RA) and Allergic Contact Dermatitis (ACD). Over the years our group has analyzed chemokine expression in lymphoid organs draining inflammatory sites, as well as in tissues from patients with RA, Ulcerative Colitis, and ACD in order to determine which ligand/receptor pairs are playing a major role. Studying the inflammatory microenvironment, it becomes crucial to evaluate not only the expression levels of the chemokine receptor agonists, but also to examine their posttranslational processing, and their interactions with inflammatory proteins released during inflammation, like PGE_2 or HMGB1. We are analyzing the cellular composition as well as the chemokine expression profile in secondary lymphoid organs draining distinct inflammatory sites. In addition we are studying the modulation of chemokine activities induced by proteins which are released in inflammation and autoimmunity.
This project is done in collaboration with Costantino Pizzalis, University of London (UK), Federica Sallusto, IRB, Bellinzona (CH) and Marco Bianchi, San Raffaele Institute, Milan (IT).

» ***Manzo A, et al.*** *Am J Pathol. 2007;* 171:1549-1562.

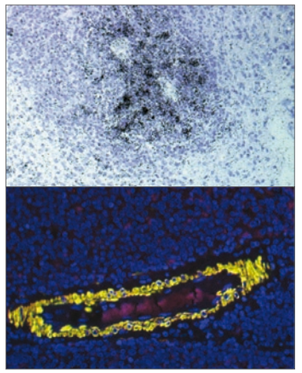
» ***Manzo A, et al.*** *Arthritis Rheum. 2008; 58:3377-3387.*

Chemokines in tumors

Daniel Venetz, Maria Gabriela Danelon and Mariagrazia Uguccioni

» *Vermi W, et al.* *J Pathol. 2008;* 216:356-364.

There are clear indications for a role of chemokines in tumor biology. Our group study the expression and activities of chemokines in sarcomas and in nodal and extranodal lymphomas. Large B cell lymphomas are a heterogeneous group accounting for about 40% of adult non-Hodgkin lymphomas. Primary central nervous system lymphomas (PCNSLs) are aggressive malignancies confined to the central nervous system, mostly of diffuse large B cell histotype. Despite improved understanding of the malignant B cell phenotype, the tumor microenvironment and the response of the adaptive immunity against PCNSLs have been neglected so far. We have investigated the tumor infiltrating lymphocytes (TILs), the expression of different lymphocyte chemoattractants, and the positioning of malignant B cells within the tumor in 23 cases of PCNSLs. Activated $CD8^+$ T cells represent the majority of TILs, and tend to accumulate in perivascular areas. Their localization and density correlates with the expression of the CXC chemokine ligand (CXCL)9, which is transcribed and translated by perivascular macrophages and pericytes around small and intermediate size vessels. CXCL9 can act on circulating T as well as malignant B cells via CXCR 3, and we show that it can synergize with CXCL12 to induce migration of lymphoma cells via CXCR4. Our findings provide molecular basis for classical histological features in PCNSL. Perivascular CXCL9 and CXCL12 expression can regulate recruitment of adaptive immune effectors to PCNSLs and further modulate angiocentric positioning of malignant B cells in the perivascular cuff.



↖ ***CXCL9 mRNA expression in perivascular area of PCNSL.*** *In situ hybridization performed on paraffin embedded tissue. Black, CYCL9 mRNAs. Magnification 20x.*

← ***CXCL9 expression in smooth muscle cells in PCNSL endothelial area.*** *Double immunofluorescence performed on paraffin-embedded tissue. Yellow, $CXCL9^+$ smooth muscle cells. Magnification 20x.*

Chemokines and chemokine receptors in infectious disease

Valentina Cecchinato and Mariagrazia Uguccioni

» *Stahl-Henning C, et al.* *Plos Pathogens 2009 5:e1000373*

More than 25 years after the discovery of human immunodeficiency virus (HIV) as the causative agent of AIDS, the mechanisms governing pathogenesis and disease progression are still not fully understood. Indeed, a progressive impairment of the immune system, with alterations that affect both innate and adaptive immunity, characterizes the infection with HIV-1 in humans and with simian immunodeficiency virus (SIV) in macaques. It has been proposed that a state of chronic immune activation contributes to loss of CD4 T cells and to alterations of immune responses, ultimately leading to disease progression. The loss of $CD4^+CCR5^+$ T cells in the gut associated lymphoid tissue (GALT) has been well documented both in natural host and in pathogenic models of SIV infection. A decrease in the

frequency of Th17 cells, a newly discovered subset of effector T cells involved in the immune response against extracellular bacteria, has been recently described in the mucosa of SIV infected animals. Nevertheless the migratory capacity of this T cell subpopulation has not been investigated so far. Chemokines are important mediators of leukocyte trafficking and function, and deregulation of their expression might contribute in part to the pathogenesis of HIV-1/SIV infection. We investigate the mechanisms that mediate Th17 cell trafficking and activities at mucosal sites together with their decrease in frequency during HIV/SIV infection in order to better understand the pathogenesis of AIDS, in view of generating efficient vaccines. In addition, we are pursuing our studies on the long-term effects of vaccines against the immunodeficiency viruses in the frame of two EU funded projects.

Details from July 2007 until June 2009

Funding

- *Swiss National Science Foundation*
  Impact of multiple chemokine expression in human disease
  3100A0-104237.1 / 2004-2007
- *Swiss National Science Foundation*
  Impact of multiple chemokine expression in human disease
  3100A0-118048.1 / 2008-2011
- *European Union*
  MAIN: Targeting cell migration in chronic inflammation
  FP6-NoE LSHG-CT-2003-502935, BBW-03.0441-1 / 2003-2008
- *European Union*
  TIP-VAC: Explaining and improving efficacy of targeted immunodeficinecy virus-like particles vaccines against AIDS
  FP6 – LSHP-CT-2004-012116 / 2005-2007
- *European Union*
  DEC-VAC: Development of a dendritic cell-targeted vaccine against AIDS
  FP6 – LSHP-CT-2005-018685 / 2005-2010
- *European Union*
  INNOCHEM: Innovative chemokine-based therapeutic strategies for autoimmunity and chronic inflammation
  FP6 – LSHP-CT-2005-518167 / 2005-2010
- *San Salvatore Foundation*
  Chemokine expression in extranodal lymphomas
  2006-2010
- *Max Cloetta Foundation*
  Student Fellowship to Daniel Venetz
  Molecular and biological mechanisms of cell trafficking in lymphoid organs. Involvement of chemokines in homeostasis and tumor growth
  2006-2009
- *European Union*
  Marie Curie Intra-European Fellowship to Valentina Cecchinato
  MD-THIV: Migration and Differentiation of Th17 in HIV/SIV infection
  FP7 - PIEF-235200 / 2009-2011

Collaborations

- *Francesco Bertoni*
  Oncology Institute of Southern Switzerland (IOSI), Bellinzona (CH)
- *Marco E. Bianchi*
  San Raffaele Institute, Milan (IT)
- *Ralf Ignatius*
  Institute of Tropical Medicine, Charité – University Medicine, Berlin (DE)
- *Massimo Locati*
  Clinical Institute Humanitas, Milan (IT)
- *Luca Mazzucchelli*
  Cantonal Institute of Pathology, Locarno (CH)
- *Marc Parmentier*
  Université Libre de Bruxelles (BE)
- *Costantino Pitzalis*
  William Harvey Research Institute, University of London (UK)
- *Maurilio Ponzoni and Claudio Doglioni*
  San Raffaele Institute, Milan (IT)
- *Christiane Stahl-Henning*
  German Primate Center, Goettingen (DE)
- *Ralph Steinman*
  Rockefeller University, New York, NY (US)
- *William Vermi*
  University of Brescia, Brescia (IT)

Visiting Scientists

- *Antonio Manzo*
  William Harvey Research Institute, University of London (UK)
- *Eleonora Olivotto*
  University of Bologna (IT)

Publications

- ***CCL21 expression pattern of human secondary lymphoid organ stroma is conserved in inflammatory lesions with lymphoid neogenesis.***
  Manzo A, Bugatti S, Caporali R, Prevo R, Jackson D G, Uguccioni M, Buckley C D, Montecucco C, Pitzalis C.
  Am J Pathol. 2007;171:1549-1562.
- ***Mature antigen-experienced T helper cells synthesize and secrete the B cell chemoattractant CXCL13 in the inflammatory environment of the rheumatoid joint.***
  Manzo A, Vitolo B, Humby F, Caporali R, Jarrossay D, Dell'accio F, Ciardelli L, Uguccioni M, Montecucco C, Pitzalis C.
  Arthritis Rheum. 2008;58:3377-3387.
- ***Identification of CXCL13 as a new marker for follicular dendritic cell sarcoma.***
  Vermi W, Lonardi S, Bosisio D, Uguccioni M, Danelon G, Pileri S, Fletcher C, Sozzani S, Zorzi F, Arrigoni G, Doglioni C, Ponzoni M, Facchetti F.
  J Pathol. 2008;216:356-364.
- ***Synergy-inducing chemokines enhance CCR2 ligand activities on monocytes.***
  Kuscher K, Danelon G, Paoletti S, Stefano L, Schiraldi M, Petkovic V, Locati M, Gerber B O, Uguccioni M.
  Eur J Immunol. 2009;39:1118-1128.
- ***Synthetic double-stranded RNAs are adjuvants for the induction of T helper 1 and humoral immune responses to human papillomavirus in rhesus macaques.***
  Stahl-Hennig C, Eisenblatter M, Jasny E, Rzehak T, Tenner-Racz K, Trumpfheller C, Salazar A M, Uberla K, Nieto K, Kleinschmidt J, Schulte R, Gissmann L, Muller M, Sacher A, Racz P, Steinman R M, Uguccioni M, Ignatius R.
  PLoS Pathog. 2009;5:e1000373.

Book chapters

Uguccioni M, Campbell JJ, Kuscher K, Kadin ME. "Lymphocytes homing and immunology of extranodal lymphoid tissue" in Text Book on Extranodal Lymphomas. Edited by F Cavalli, H Stein, E Zucca. Informa UK Ltd. 2008

Lectures and Seminars

- *The Italian Society for Clinical Immunology and Allergy (SIICA) Annual Meeting*
  Trieste (IT) / 06.06.2007
- *University of Bologna*
  Seminar "Chemokine guide leukocyte trafficking"
  Bologna (IT) / 16.06.2007
- *Italian Association of Immunogenetics and Transplantation (AIBT)*
  Rome (IT) / 25.06.2007
- *University of Brescia*
  Seminar "Natural chemokines can affect leukocytetTrafficking"
  Brescia (IT) / 19.11.2007
- *2008 International Guidelines on Rhinitis, Asthma and COPD. Global Initiatives ARIA, GINA and GOLD/ATS/ERS*
  Ferrara (IT) / 07.03.2008
- *The Swiss Society for Allergy and Immunology Annual Meeting*
  Fribourg (CH) / 16.04.2008
- *University of Tor Vergata*
  Seminar "Chemokines as potential therapeutic targets"
  Rome (IT) / 27.11.2008
- *German Primate Center*
  Seminar "Chemokines as potential therapeutic targets"
  Goettingen (DE) / 25.02.2009
- *William Harvey Research Institute, University of London*
  Seminar "Chemokine activities in inflammation and tumour"
  London (UK) / 17.04.2009
- *Istituti Ortopedici Rizzoli*
  Seminar "Chemokine as potential therapeutic targets"
  Bologna (IT) / 24.06.2009
- *Academy of Science, University of Bologna*
  Lecture "Chemokine in the immunepathogenesis of chronic inflammation and lymphomas"
  Bologna (IT) / 24.06.2009

Luca Varani
Structural Biology

Mariagrazia Uguccioni
Marcus Thelen
Federica Sallusto
Silvia Monticelli
Maurizio Molinari
Markus G Manz
Antonio Lanzavecchia
Fabio Grassi

Luca Varani

Luca Varani graduated in chemistry at the University of Milan (IT) with a thesis in structural biology. He then moved to the MRC-Laboratory of Molecular Biology, obtaining a PhD degree at the University of Cambridge (UK) in 2000. His PhD research focused on the role of RNA and protein interactions in regulation of gene expression at the post-transcriptional level, culminating in the determination of the largest NMR structure and one of only 3 RNA-protein complexes available at the time. He also contributed to show the role of RNA structure in dementia, proving the viability of RNA as a therapeutic target. After a brief spell in Florence, he moved to Stanford University (US) as a postdoctoral fellow, being awarded an "EMBO Fellowship" in 2003. At Stanford he completed the first NMR study on TCR-pMHC complexes, proposing a novel approach to the systematic characterization of protein-protein interactions. In October 2007 he joined the IRB as a Group Leader of the Structural Biology Laboratory.

Research Focus

Our group uses computational, biochemical and biophysical tools to determine the structure of proteins and to characterize their interactions with other molecules, with particular attention to antibody/antigen interactions in viral diseases. Until recently, the only tools available to investigate biomolecular structures at the atomic level were NMR and X-Ray crystallography, well established techniques where revolutionary advances are not likely to happen. On the other hand, Computational Structural Biology is a novel, exciting field with very rapid development and high expectations for the near future. We can use computers to predict individual structures (modelling) and intermolecular complexes (docking) and the speed, precision and accuracy of these predictions will increase with computational power and novel algorithms. Computer predictions, however, are not always accurate, so it is important to experimentally validate them. What has largely been missing to achieve this goal is a concerted effort by different branches of the life sciences such as biology and informatics. Here we strive for an integrated approach to merge biochemical data, experimental structural validation and computational docking in an efficient workflow, and to apply it to biologically relevant cases. The group was started in October 2007. Current projects involve the characterization of antibody-protein interactions in Dengue and Influenza virus. Pilot projects are undergoing to determine the structure of protein receptors in human Cytomegalovirus and to study interactions and synergism in chemokines.

Team

Group Leader: Luca Varani › *luca.varani@irb.unisi.ch*
Members: Luca Simonelli, *PhD student* – Zinaida Yudina, *PhD student*

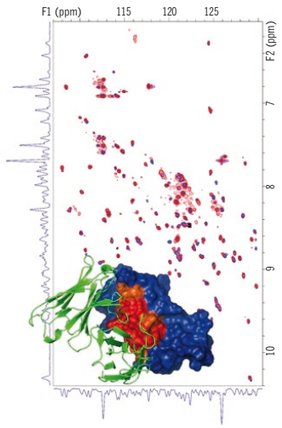
Prediction and characterization of antibody-protein interactions in Dengue virus

Luca Simonelli, Zinaida Yudina and Luca Varani

Since most current vaccines are based on the generation of neutralizing antibody responses, a better understanding of the interaction between antibodies and pathogens is expected to accelerate vaccine development. The project objective is to develop a novel, rapid, computational approach for the structural characterization of a large panel of different antibodies bound to the same antigen, with the aim of drawing general rules to be applied to the design of new drugs and vaccines. Computer predictions, however, are not always accurate, so we need to experimentally validate them and improve their accuracy. What has largely been missing to achieve this goal is a concerted effort by different branches of the life sciences such as biology and informatics. Here we propose to develop an integrated approach to merge biochemical data, experimental structural validation and computational docking methods in an efficient workflow, and to apply it to biologically relevant cases such as Dengue or Influenza Virus.

Strategy and rationale. Individuals that survive a viral infection have antibodies (Ab) capable of detecting and neutralizing subsequent attack by the same virus. Detection and neutralization are initiated by the binding of these antibodies to antigens (Ag), often viral proteins, through specific atomic interactions between the antibody and the region of the antigen that it recognizes (epitope).If we understand the biochemical rules governing antibody/antigen interactions in a given virus, then we have the molecular basis to try to design and synthesize new epitopes to be used as vaccines (prevention) or new drugs mimicking the antibodies (cure). The best way to study atomic interactions is obtaining the three-dimensional structure of Ab/Ag complexes by X-Ray crystallography: a long process with high failure rate. While this method has provided structures of specific complexes between antibodies and proteins, if we want to understand general principles of Ab/Ag recognition we need to study the binding of several different Abs to the same antigen. Indeed, studying a single complex has further limitations, since it may not thoroughly represent viruses with significant variations over time or geographical distance. We here propose to devise a novel, rapid, computational approach for the structural characterization of a large panel of different antibodies bound to the same antigen, with the aim of drawing general rules to be applied to the design of new drugs and vaccines. We might discover, for example, that all the antibodies with best neutralizing properties bind to the same epitope, which ideally we could then synthesize and use as vaccine. Conversely, should the most efficient antibodies share common chemical features, we could try to design a molecule, be it a novel compound or simply an aptly modified antibody, exploiting those features. Advances in computing power allow us to apply computational docking (the process of obtaining the structure of a complex between two molecular components) to the study of large and computational demanding Ab/Ag complexes. While computational docking is fast, the structures it provides might not be accurate, however. Here we propose to use existing programs for docking but to improve their accuracy in two ways: **1)** by using rapidly obtained experimental biochemical data both to drive and validate the computational results; **2)** by devising improved protocols specifically designed for the peculiarities of antibodies. Computational approaches shall also play a role in later stages: we might be able to design several different epitopes (or antigens) and screen in silico for their capacity to bind neutralizing antibodies, in a similar way to the in silico small-drug screening currently used in the pharmaceutical industry. Furthermore, the thermodynamic effect of chemical substitution either in the epitopes or antibodies can also be rapidly assessed with computer simulations, allowing a better understanding of the principles of Ab/Ag recognition. The results of computational docking will not be as precise as experimental structural determination, but I believe that we can learn more by studying general trends in a large panel of slightly inaccurate structures than a single complex with high precision and accuracy. It is also undeniable that computational methods will become more accurate, rapid and economical over the years, improving the relevance of a computational approach to vaccine design. The approach can be applied to virtually any viral disease. As a proof of concept we initially test it on Dengue, where virus variations pose a particular challenge to vaccine design and subsequently apply it to other viruses such as Influenza and HIV.

*Proof of concept: Dengue virus.*Dengue virus causes an estimated 100 million cases of Dengue fever each year, mostly in poor, tropical countries. The task of finding a cure has been hampered by the presence of four different Dengue serotypes and by a poorly understood process unique in human medicine, Antibody Dependent Enhancement (ADE): antibodies raised against a previous Dengue infection facilitate subsequent infection by a different serotype and lead to Dengue Hemorrhagic Fever (DHF), a lethal form of the disease. This feature complicates the task of finding a vaccine against Dengue, since a vaccine that would not protect equally against all four existent serotypes would actually contribute to the emergence of DHF. This proposal aims at furthering our understanding of the molecular basis of neutralization and ADE by characterizing and comparing the binding of several antibodies to the viral surface protein from different serotypes. Understanding the origins of ADE could spark new ideas to overcome the problem, with obvious implications for the development of a cure against Dengue virus. We will isolate several antibodies from donors that have recovered from Dengue, characterize their neutralizing and enhancing properties and obtain the structure of their complexes with Dengue E protein, using NMR and other rapidly obtainable experimental data to drive and validate our biocomputational models. Schematically, we will isolate antibodies that bind to domain III of E protein, a known antigen, from different serotypes, classify them according to their ability to neutralize and/or enhance different serotypes, build computational homology models of the antibodies according to available techniques, obtain computational models of the Abs bound to domain III using an aptly modified version of the program "Rosetta-docking", using the available



x-ray structure of domain III and the generated models of antibodies as starting material. Computational docking will be driven and validated by experimental data such as NMR chemical shift mapping, viral mutagenesis and competition assays. Being able to rationally modify an existing antibody to change its binding preference from one serotype to another would be outstanding. Regardless, in the following years we will try to obtain and study the models of tenths of antibodies bound to each serotype. Should our approach prove to be valid for Dengue virus, it could then be applied to any other viral disease, with particular relevance for those that mutate often (eg HIV or Influenza) and for which a systematic approach would prove more useful.

This project is done in collaboration with Antonio Lanzavecchia and Martina Beltramello, IRB, Bellinzona (CH).

←***Computational docking model of a neutralizing antibody on the surface protein of Dengue virus.*** *The protein is shown in blue and the Ab in green; the NMR-derived epitope is in red and orange. Blue peaks are from the NMR spectrum of the free protein and red peaks from its complex with the antibody.*

Details from July 2007 until June 2009

Collaborations

- *Swiss Supercomputer Center (CSCS)*
  Manno (CH)
- *Luigi Calzolai*
  European Union Joint Research Center,
  Ispra (IT)
- *NMR center MRC-NIMR*
  Mill Hill, London (UK)
- *Michele Parrinello*
  ETHZ/USI, Lugano (CH)
- *Anna Tramontano*
  Università degli Studi La Sapienza, Rome (IT)

Visiting Scientists

- *Tiziana Lischetti*
  Università degli Studi dell'Insubria, Varese (IT)

Lectures and Seminars

- *MRC-NIMR*
  Structural Biology Seminar series "Rapid detection and characterization of TCR/pMHC interactions"
  London (UK) / 20.09.2007
- *XXXVII National Congress on Magnetic Resonance*
  Verbania (IT) / 13.09.2007
- *CHUV (Centre Hospitalier Universitaire Vaudois)*
  Seminar "Rapid structural characterization of antibody/antigen complexes"
  Lausanne (CH) / 20.11.2008
- *University of Kent*
  Seminar "A method for detection and characterization of TCR/pMHC interactions"
  Chatham (UK) / 30.04.2008
- *Università degli Studi dell'Insubria*
  Seminar "Rapid detection and characterization of TCR/pMHC interactions"
  Busto Arisizio (IT) / 29.01.2008
- *Institute for Health and Consumer Protection; Joint Research Center*
  Seminar "Rapid structural characterization of antibody-antigen complexes through experimentally validated computational docking"
  Ispra (IT) / 15.06.2009
- *University of Italian Switzerland (USI)*
  Seminar "Rapid structural characterization of antibody-antigen complexes through experimentally validated computational docking"
  Lugano (CH) / 22.06.2009

SECTION 2

PhD PROGRAM

INDEX SECTION 2 - PhD PROGRAM

Tito Calì

Tuning endoplasmic reticulum associated degradation

Supervisor: Maurizio Molinari // Co-referees: Peter Buetikofer and Dirk Dobbelaere

PhD Program, Graduate School for Cellular and Biomedical Sciences, University of Bern

Proteins expressed in the endoplasmic reticulum (ER) are covalently modified by co-translational addition of pre-assembled core glycans (glucose3-mannose9-N-acetylglucosamine2) to asparagines in Asn-X-Ser/Thr motifs. N-Glycan processing is essential for protein quality control in the ER. Cleavages and re-additions of the innermost glucose residue prolong folding attempts in the calnexin cycle. Progressive loss of mannoses is a symptom of long retention in the ER and elicits preparation of terminally misfolded polypeptides for dislocation into the cytosol and proteasome-mediated degradation. The ER stress-induced protein EDEM1 accelerates ER-associated degradation (ERAD) by extracting non-native glycopolypeptides from the calnexin chaperone system, by accelerating de-mannosylation of terminally misfolded glycoproteins and by inhibiting formation of covalent aggregates. The intralumenal level of EDEM1 can be increased upon activation of transcriptional programs under conditions of ER stress or of enhanced cargo load. However under normal growth conditions, the intralumenal level of EDEM1 must be low to prevent premature interruption of ongoing folding programs. Tito Calì found that, in unstressed cells, EDEM1 is segregated from the bulk ER and is rapidly degraded. Cells with defective EDEM1 turnover show enhanced ERAD activity and are characterized by impaired capacity to efficiently complete maturation of model glycopolypeptides. We define as *ERAD tuning* the mechanisms operating in the mammalian ER at steady state to offer kinetic advantage to folding over disposal of unstructured nascent chains by selective and rapid degradation of ERAD regulators.

» ***Olivari S. et al.***
Biochem. Biophys. 2006; Res. Commun. 349, 1278-1284.

» ***Calì T. et al.***
Biochem. Biophys. 2008; Res. Commun. 371, 405-410.

» ***Calì T. et al.***
Progress in Nucl. Ac. Res. and Mol. Biol.
2008; 83, 135-179.

Anna Casati

Hematopoiesis during T cell mediated inflammation

Supervisor: Fabio Grassi

PhD Program in Biotechnology Applied to Medical Sciences, University of Milan (IT)

Activation and repression of specific genetic programs determine lineage commitment and differentiation of haematopoietic stem cells. Whether and which instructive or cell autonomous processes determine the triggering of such programs would contribute to the definition of the developmental biology of the immune system. In pathological conditions perturbation of the homeostasis between cells of different lineages or increased demand of particular subset/s could modify steady state haematopoietic programs. Haematopoietic stem cells (HSCs) give rise to all type of blood cells. Long-term (LT)-HSCs represent a minute self-renewing and quiescent cell population with the potential to enter cell cycle, differentiate into progenitors belonging to different cell lineages and replenish the cellular component of the blood. Multiple intermediates with distinct developmental potentials and specific molecular signatures have been defined to date. Haematological alterations are counteracted by bone marrow adaptation to peripheral needs without depletion of the stem cell pool. However, regulatory networks and peripheral cells controlling haematopoiesis in pathological conditions are poorly defined. Common myeloid progenitor (CMP) and common lymphoid progenitor (CLP) represent the earliest branch point between the myeloid and lymphoid lineages. Further commitment of CMP to either megacaryocyte/erythrocyte precursor (MEP) or granulocyte/monocyte precursor (GMP) is mutually exclusive.

To understand the effects of immunopatologically activated T cells on hematopoiesis, we characterized cellular composition in the bone marrow of two murine models of T cell dependent immunopathology, namely calreticulin-deficient fetal liver chimeras ($crt^{-/-}$ FLC) in lymphopenic hosts and inflammatory bowel disease (IBD) induced in lymphopenic mice by adoptive transfer of naïve CD4 cells. $Crt^{-/-}$ T cells display an altered regulation of the activation machinery with protracted nuclear translocation of nuclear factor of activated T cell (NFAT) and activation of the MAPK pathway. This results in lower threshold of T cell activation and hyper responsiveness upon antigen encounter. $Crt^{-/-}$ FLC develop a T cell dependent inflammatory disease characterized by severe blepharitis and alopecia.

In the bone marrow of these two experimental models of T cell mediated tissue inflammation we observed an altered B cell lymphopoiesis with a selective repression of pro-B, pre-B and immature B cell subsets. In contrast, the relative representation of the mature B cell compartment in the bone marrow was significantly increased. We correlated this increase to enhanced secretion of macrophage migration inhibitory factor (MIF) by perisinusoidal dendritic cells in the bone marrow and established a causal relationship of mature B cell increase with MIF secretion by treating mice with IBD with a selective MIF antagonist.

There is an intimate and highly regulated link between primary leucopoiesis and inflammation that acts to expand the production of granulocytes and monocytes in response to infection. During infection, acute monocyte and granulocyte responses are crucial for host protection, and, in contrast to lymphocytes, most mature granulocytes and monocytes are short lived. To analyse at which level immunopathological T cell activation affected hematopoiesis, we scored hematopoietic progenitors and defined the reciprocal relationship between immature B cell progenitors and granulocyte/monocyte precursors. The expansion of the latter was dependent on effector/memory T cells peripheral expansion and tissue damage. Toll-like receptors are activated by pathogen-associated molecular pattern (PAMP) and are expressed on HSCs. TLR agonists can modulate and influence HSCs proliferation and fate determination. We characterized the responsiveness and skewing toward granulopoiesis of HSCs by Toll-like receptor ligands. Moreover, we assessed the role of ATP as host-derived danger signal released after cell damage on HSC proliferation and differentiation toward the granulocyte/monocyte path through purinergic P2X receptors stimulation. The administration of a P2X antagonist, oxidized ATP, significantly reduced HSC expansion. In summary, the data contributed by this study provide a comprehensive picture of the impact of T cell-mediated immunopathology on hematopoiesis.

» ***Marrella V, et al.*** *J Clin Invest. 2007;* 117:1260-1269.

» ***Schenk U, et al.*** *Sci Signal. 2008;* 1:ra6.

Davide Corti

Analysis of the human memory B cell repertoire against infectious pathogens and isolation of broadly neutralizing human monoclonal antibodies

Supervisor: Antonio Lanzavecchia // Co-referee: Clemens Dahinden
Interfaculty PhD studies, University of Bern

Human memory B cells represent an accessible source of all the antibody specificities produced during the lifespan of an individual. The analysis of the B cell memory repertoire against infectious pathogens is of utmost significance: i) to document dynamic changes following infection or vaccination, ii) to identify and isolate monoclonal antibodies with useful properties and iii) to identify antigenic determinants targeted by neutralizing antibodies in complex or variable pathogens. In the first part of my work I developed a culture system for antigen specific memory B cell repertoire analysis (AMBRA method) by culturing total peripheral blood mononuclear cells with TLR7/8 agonist R848 and IL-2 for the selective activation and differentiation of human memory B cells. This method was used to establish frequency and fine specificity of memory B cells for a variety of human pathogens and their kinetics following infection or vaccination. In the second part of my work I used an improved method of memory B cell immortalization to isolate human monoclonal antibodies that would be capable of broadly neutralizing viral targets. Several SARS-CoV specific antibodies isolated from a recovered patient were characterized for their fine specificity and for their capacity to neutralize human and zoonotic SARS-CoV isolates. More recently I used this method to isolate from non-clade B infected individuals human monoclonal antibodies that neutralize HIV-1. This work led to the isolation of several neutralizing antibodies, some of which showed broad spectrum of neutralizing activity in vitro against several HIV-1 isolates. The isolation of broadly neutralizing monoclonal antibodies is relevant not only for serotherapy, but also for "analytic vaccinology" where such antibodies are used as probes to identify conserved epitopes that may be properly formulated as vaccines.

» ***Zhu Z, et al.***
Proc Natl Acad Sci USA. 2007;
104:12123-12128.

» ***Lanzavecchia A, et al.***
Curr Opin Biotechnol. 2007;
18:523-528.

» ***Rockx B, et al.***
J Virol. 2008;
82:3220-3235.

» ***Cocco M, et al.***
Am J Pathol 2008;
173:1369-1378.

» ***Pinna D, et al.***
Eur J Immunol 2009;
39:1260-1270.

Denise Ferrera

Subcellular microdomains in T cell development

Supervisor: Fabio Grassi // Second Supervisor: Ruggero Pardi
University "Vita e Salute" San Raffaele, Milan (IT)

The aim of my project was to elucidate whether plasma membrane rafts play a role in pre-TCR signaling. Ligand-independent oligomerization of the pre-TCR in the plasma membrane promotes β-selection of thymocyte. However, whether pre-TCR affinity for rafts is important in initiation of signaling by the pre-TCR is controversial. Calnexin (CNX)/CD3 complexes are expressed in the plasma membrane of recombinase-deficient thymocyte and their crosslinking mimics pre-TCR signaling. We have shown that, analogously to the pre-TCR, surface CNX/CD3 prominently accumulated into lipid rafts upon cross-linking. We have designed and expressed different mutant CNX isoforms to shuttle CD3 to rafts with different efficiencies. Strong but not weak translocation of CNX/CD3 into rafts determined pre-TCR-like signaling and simulated β-selection, demonstrating that rafts act as repositories of signaling components relevant for pre-TCR signaling. Inclusion of cytoplasmic tail from pre-Tα (pTα) chain in place of CNX cytoplasmic tail increased pre-TCR signaling efficiency. Our work indicates that signaling by oligomerized pre-TCR initiates in lipid rafts and supports the view that β-_selection is implemented by pTα cytoplasmic tail.

» ***Porcellini S, et al.***
J Exp Med. 2006;
203:461-471.

» ***Ferrera D, et al.***
Eur J Immunol. 2008;
38:1148-1156.

Rebekka Geiger

Analysis of the human naive T cell repertoire and identification of a novel T helper subset

Supervisor: Federica Sallusto // Co-referee: Hans-Uwe Simon // Mentor: Thomas Brunner
Graduate School for Cellular and Biomedical Sciences, University of Bern

One hallmark of the immune system is its capability to respond to a large variety of different antigens. T cells are characterized by a highly diverse repertoire of antigen-receptors and are one of the main players in adaptive immunity. During a primary response, cells are recruited from the naive pool and expand and differentiate to fight the invading pathogen. Upon re-exposure with the same antigen, existing memory cells get reactivated and respond faster to clear the pathogen. While several methods are available to study the antigenic repertoire of human memory T cells, to date there is no method available to analyze antigen-specific naive T cells, due to their low frequency and high activation threshold. We report a novel method that, after polyclonal amplification of naive T cells, permits to assess the frequency of human naive T cells specific for antigens of any source and complexity, which are naturally processed and presented by antigen-presenting cells (APCs). Using this method on naive and memory T cells isolated from the same donors, we found that antigen-specific naive T cells display a wide range of functional avidities, whereas memory T cells specific for the same antigen are enriched for cells displaying a high functional avidity. The method described may find applications for rapid identification of antigenic sites in vaccine candidates, for testing antigenicity of therapeutic proteins, drugs and chemicals and for generation of antigen-specific T cell clones of desired specificity and function for adoptive cellular immunotherapy. Recognition of components of invading pathogens by antigen-specific cells initiates the immune response. However to effectively protect the body from pathogen spreading, the immune response has to be tailored to the type of infectious agent. At present, different lineages of T helper cells have been identified that are characterized by the production of distinct cytokines. Th1 cells produce IFN-γ and activate macrophages, thus mediating protection against intracellular pathogens such as Mycobacterium tuberculosis. Th2 cells produce IL-4, IL-5 and IL-13 and mediate protection against extracellular parasites, whereas Th17 cells produce IL-17A, IL-17F and IL-22 and are important in responses against fungi and bacteria. Th1, Th2 and Th17 cells cause also immune pathologies, such as autoimmunity and allergy. We describe a novel population of T helper cells in peripheral blood, which is characterized by production of IL-22, but not IL-17 nor IFN-γ and low to undetectable levels of the Th17-specific transcription factor RORγt and the Th1 specific transcription factor T-bet. This population is identified by expression of the skin homing molecules CCR10, CCR6 and CCR4 and can be generated in vitro by plasmacytoid dendritic cells (pDCs) through production of IL-6 and TNF-α. This novel subset of T helper cells, that we have operationally defined Th22, may play a role in immune surveillance of the skin and participate in skin pathologies such as psoriasis and atopic dermatitis.

» ***Geiger R, et al.***
J Exp Med. 2009; 206:1525-1534.

» ***Duhen T, et al.***
Nat Immunol. 2009; 10:857-863.

Katrin Kuscher

Synergy-inducing chemokines enhance CCR2 ligand activity on monocytes

Supervisor: Mariagrazia Uguccioni // Co-referee: Bernhard Moser // Mentor: Thomas Brunner
Graduate School for Cellular and Biomedical Sciences, University of Bern

The migration of monocytes to sites of inflammation is largely determined by their response to chemokines. While the chemokine specificities and expression patterns of chemokine receptors are well defined, it is still a matter of debate how cells integrate the messages provided by different chemokines that are concomitantly produced in physiological or pathological situations in vivo. Katrin Kuscher during her PhD thesis work has presented evidence for a regulatory mechanism of human monocyte trafficking. Monocytes can integrate stimuli provided by inflammatory chemokines in the presence of homeostatic chemokines. In particular, migration and cell responses could occur at much lower concentration of the CCR2 agonists, in the presence of chemokines (CCL19 and CCL21) that per se do not act on monocytes. Binding studies on CCR2+ cells showed that CCL19 and CCL21 do not compete with the CCR2 agonist CCL2. More over, the presence of CCL19 or CCL21 could influence the degradation of CCL2 and CCL7 on cells expressing the decoy receptor D6. These findings have disclosed a new scenario to further comprehend the complexity of chemokine-based monocyte trafficking in a vast variety of human inflammatory disorders.

» ***Kuscher K, et al.***
Eur J Immunol. 2009; 39:1118-1128.

Debora Pinna

Dynamics, specificities and cross-reactivity of the human B cell response to Influenza virus

Supervisor: Antonio Lanzavecchia // Co-referee: Fabio Grassi
PhD Program Molecular Medicine, Basic and Applied Immunology. University "Vita e Salute" San Raffaele, Milan (IT)

The aim of my thesis work was the study of the humoral response to viral infections and vaccination with particular attention to the maintenance and composition of the human B cell memory repertoire. The analysis of the B cell memory repertoire against infectious pathogens is of utmost significance: i) to document dynamic changes following infection or vaccination, ii) to identify and isolate monoclonal antibodies with useful properties and iii) to identify antigenic determinants targeted by neutralizing antibodies in complex or variable pathogens. In the first part of my thesis I describe a new method that I developed to study, at a monoclonal level, antigen specificity and composition of the human B cell memory repertoire. Total peripheral blood mononuclear cells (PBMC) were cultured in the presence of R848, the agonist for Toll Like Receptors (TLRs) 7 and 8 and of high doses of IL-2. These simple culture conditions were sufficient and selective to trigger memory B cell expansion with a cloning efficiency ranging from 26 to 64%. This method was used to establish frequency and fine specificity of memory B cells for a variety of human pathogens and their kinetics following infection or vaccination. In the second part of my work I tried to characterize the heterosubtypic antibody response to the classical Influenza vaccine. Recent reports describe the existence of heterosubtypic antibodies from human phage libraries thus demonstrating the existence of conserved epitopes between hemagglutinin (HA) of different subtypes. To investigate whether this kind of response could be elicited by the seasonal Influenza vaccine I have analyzed the response of several immunized donors with the purpose to identify a possible heterosubtypic antibody neutralizing response. Using an improved method of memory B cell immortalization, I was able to isolate a panel of human monoclonal antibodies with breath of neutralizing reactivity against different human H5N1 isolates, as well as H1N1. These antibodies were also protective in an in vivo setting.

» ***Pinna D, et al.*** *Eur J Immunol. 2009; 39*:1260-1270.

Omar Vanoni

Protein Folding in the ER: the fate of β-secretase N-glycosylation mutants

Supervisor: Maurizio Molinari // Co-referees: Ari Helenius and Ruedi Glockshuber
PhD Program, ETH Zurich

The endoplasmic reticulum (ER) houses a quality control machinery for newly synthesized proteins destined to the cellular plasma membrane, to endo- and exocytic compartments and to the extracellular space. The quality control insures, with rare exceptions, that only native proteins are released from the ER. Folding intermediates, orphan subunits of oligomeric complexes and misfolded polypeptides are retained in the compartment. Cell and organism homeostasis depends on the regulated balance between transport of native proteins to the site of destination and disposal of terminally misfolded structures. To determine how protein-bound oligosaccharides determine the fate of newly synthesized polypeptides and how folding and degradation machineries compete during protein biogenesis, Omar determined the fate of soluble forms of Beta-site APP cleaving enzyme 1 (BACEs) with 4, 3, 2, 1 and 0 N-glycosylation sites expressed in mammalian cells with active and inactive proteasomes. Analysis of the fate of the five BACEs variants revealed a direct correlation between the number of N-glycans displayed on the nascent polypeptide chains and folding efficiency in the calnexin chaperone system, folding rate and secretion. Addition of 1 to 4 N-glycans progressively enhanced the dissociation rate from BiP and reduced the propensity of newly synthesized BACEs to enter aberrant soluble and insoluble aggregates. Finally, inhibition of the proteasome increased the yield of active BACEs secreted from cells. This suggested that a fraction of BACEs undergoes premature degradation that decreases the amount of the enzyme that can attain the native structure.

Omar also gave an essential contribution in the characterization of the UDP-glucose:glycoprotein glucosyltransferase (UGT1), an ER resident protein that insures retention in the ER lumen of misfolded polypeptides. Omar discovered that the UGT1 is a central player of glycoprotein quality control in the ER. It re-glucosylates non-native glycopolypeptides, thus inhibiting their release from calnexin, a sugar-binding ER chaperone. The finding that persistently misfolded glycoproteins were eventually released from calnexin and entered disulfide-bonded aggregates associated with BiP/GRP78 led to propose that retention-based ER quality control consists in two phases involving two distinct chaperone complexes, the calnexin and the BiP system.

» ***Molinari M. et al.*** Mol. Cell 20 (2005); 503-512.

» ***Vanoni O. et al.*** Mol. Biol. Cell 19 (2008), 4086-4098.

» ***Calì T. et al.*** Progress in Nucl. Ac. Res. and Mol. Biol. (2008); 83, 135-179.

SECTION 3

PhD LECTURE COURSES & SEMINARS

INDEX SECTION 3 - PhD LECTURE COURSES & SEMINARS

The IRB PhD Lecture Course is supported by the Gustav & Ruth Jacob Foundation

PhD Lecture Course 2007 - 2008

- *Michael McHeyzer-Williams*
  "Antigen specific memory B cell development"
  The Scripps Research Institute, La Jolla, CA (US) / 10.10.2007
- *Gisou Van der Goot*
  "Bacterial toxins"
  Global Health Institute, École Polytechnique Fédérale de Lausanne (CH) / 31.10.2007
- *Oreste Acuto*
  "Signal transduction in T lymphocytes"
  Sir William Dunn School of Pathology, University of Oxford (UK) / 06.11.2007
- *Eric Vivier*
  "NK cell biology"
  Centre d'Immunologie de Marseille-Luminy, CNRS-INSERM-Université de la Méditerranée Campus de Luminy, Marseille Cedex (FR) / 14.11.2007
- *Hanno Langen*
  "Proteomics in immunology and oncology"
  Center for Medical Genomics, F. Hoffmann-La Roche, Basel (CH) / 17.01.2008
- *Michael S. Neuberger*
  "Antibody diversification and selection"
  Trinity College Cambridge (UK) / 19.02.2008
- *Peter Lansdorp*
  "Telomers and telomerase biology"
  The University of British Columbia, Terry Fox Laboratory, Vancouver (CA) / 26.02.2008
- *Anna Tramontano*
  "Antibody structure"
  University of Rome"La Sapienza", Rome (IT) / 04.03.2008
- *Peter Krammer*
  "Apoptosis in the immune system"
  German Cancer Research Center, Heidelberg (DE) / 03.04.2008
- *Timm Schroeder*
  "Signaling in hematopoiesis"
  Institute of Stem Cell Research, Munich/Neuherberg (DE) / 15.04.2008
- *Witold Filipowicz*
  "Post-transcriptional gene regulation by microRNA"
  Friedrich Miescher Institute for Biomedical Research, Basel (CH)/ 08.05.2008
- *Dimitris Kioussis*
  "Lymphoid organogenesis"
  MRC National Institute for Medical Research, London (UK) / 20.05.2008
- *Brigitta Stockinger*
  "Inglammatory T cells"
  MRC National Institute for Medical Research, London (UK) / 27.05.2008
- *Sergio Grinstein*
  "Phagocytosis and phagosome biology"
  The Hospital for Sick Children, Toronto (CA) / 13.06.2008

PhD Lecture Course 2008 - 2009

- *Antonio Alcamí*
  "Modulation of immunity by virus-encoded cytokine and chemokine decoy receptors"
  Centro de Biología Molecular Severo Ochoa, Campus de Cantoblanco, Madrid (ES) / 05.11.2008
- *Jacques Banchereau*
  "Harnessing dendritic cells for a better human health"
  Baylor Institute for Immunology Research, INSERM U899 – BIIR/INSERM/ANRS Center for Human Vaccines, Live Oak, Dallas (US) / 11.12.2008
- *Federico Caligaris Cappio*
  "Chronic Lymphocytic Leukemia: a tale of cellular and molecular interactions"
  Università Vita-Salute San Raffaele, Milan (IT) / 08.01.2009
- *Ana Cumano*
  "Hematopoietic stem cells"
  Istitut Pasteur, Paris (FR) / 21.01.2009
- *Klaus Rajewsky*
  "B cell development"
  Immune Disease Institute and Harvard Medical School, Boston. US / 10.02.2009

- *Ruslan Medzhitov*
  "Innate host defense pathways"
  Yale University School of Medicine, New Haven (US) / 26.02.2009
- *Jonathan W. Yewdel*
  "Presentation of viral antigens to $CD8^+$ T cells"
  National Institute of Allergy and Infectious Diseases, Bethesda (US) / 11.03.2009
- *Bernhard Moser*
  "Human γδ T cells: alternative antigen-presenting cells"
  School of Medicine, Cardiff University, Cardiff (UK) / 25.03.2009
- *Erez Raz*
  "Chemokine guided cell migration in zebrafish"
  Institute of Cell Biology, ZMBE, Münster, DE / 29.04.2009
- *Abul K. Abbas*
  "Tolerance, autoimmunity and interleukin-2"
  UCSF University of California, San Francisco (US) / 07.05.2009
- *Hiromitsu Nakauchi*
  "Molecular regulation of hematopoietic stem cell self-renewal and dormancy"
  The Institute of Medical Science, The University of Tokyo (JP) / 28.05.2009
- *Michael D. Cahalan*
  "Cellular and molecular choreography of lymphocyte activation"
  School of Medicine, University of California, Irvine (US) / 19.06.2009

Seminar Program from July 2007 until June 2009

- *Daniele Gaudiosi*
  "Chromatin-associated functions of the CDK inhibitor p27KIP1"
  European Institute of Oncology, Milan (IT) / 23.08.2007
- *Michael S Diamond*
  "Innate and adaptive immune system protection against West Nile virus"
  Washington University School of Medicine, St. Louis (US) / 31.08.2007
- *Miriam Merad*
  "Role of Dendritic Cells in Transplant Immunity"
  Mount Sinai School of Medicine, New York (US) / 05.09.2007
- *Christina Zielinski*
  "Intrinsic T cell defects in a murine model of lupus erythematosus"
  University of Tuebingen (DE) / 06.09.2007
- *Alessandra Giorgetti*
  "Expression of Id1 gene in Circulating Hemangiogenic Cells Correlates with Tumorigenesis"
  Kedrion s.p.a., Castelvecchio Pascoli, Barga (IT) /14.09.2007
- *Annette Oxenius*
  "Immune control of Legionella pneumophila infection"
  ETH Zurich, Institute of Microbiology, Zurich (CH) / 17.09.2007
- *Urs Karrer*
  "Influence of persistent viral infections on immune senescence"
  University Hospital Zurich (CH) / 17.09.2007
- *Emmanuel Delamarche*
  "H11250N1 – Using IBM technology for checkmating a pandemic"
  IBM Zurich Research Laboratory, Zurich (CH) / 09.10.2007
- *Paul Parren*
  "Anti-inflammatory Activity of IgG4 antibodies by Dynamic Fab Arm Exchange"
  Research & Technology Genmab B.V., Utrecht (NL) / 07.11.2007
- *Maurizio Ceppi*
  "Fine-tuning of Dendritic Cells activation: lost in translation?"
  Centre d'Immunologie INSERM-CNRS, Marseille (FR) / 09.11.2007
- *Daniela Bossi*
  "Identification of the haematopoietic target cell of acute promyelocytic leukaemia (APL) in a PML-RARa transgenic mouse model"
  European Institute of Oncology, Milan (IT) / 30.11.2007
- *Jaap Goudsmit*
  "Cross-reactive human monoclonal antibodies to avian influenza: analysis of the human B cell reservoir"
  Academic Medical Center-University of Amsterdam (NL) / 11.12.2007
- *Thomas Calzascia*
  "Boosting T cell immunity to cancer and chronic viral infections using IL-7 therapy"
  Campbell Family Institute for Breast Cancer Research, Ontario Cancer Institute, Toronto (CA) / 20.12.2007
- *Dorothée Duluc*
  "LIF and IL-6 induce Tumor-Associated Macrophage like-cells"
  University Hospital of Angers (FR) / 07.02.2008
- *Sandro Carrara*
  "Recent and Innovative Nanotechnology Advancements in Biosensors"
  Swiss Federal Insitute of Technology (EPFL), Lausanne (CH)/ 15.02.2008
- *Hedda Wardemann*
  "Recombinant monoclonal antibodies cloned from single human B cells"
  Max-Planck Institute for Infection Biology, Berlin (DE) / 25.02.2008
- *Mihaela Zavolan*
  "Combining computational with experimental approaches to identify miRNA and RNA-binding protein targets"
  Biology Center, University of Basel (CH) / 29.02.2008

- *Fabrizio Chiti*
  "The determinants of protein aggregation: applications to animal models and to the bioinformatic analysis of the human proteome"
  University of Florence (IT) / 17.03.2008
- *Olivier Michielin*
  "Molecular modelling of protein: applications to cancer therapies"
  Swiss Institute of Bioinformatics and the Ludwig Institute of Cancer Research, University of Lausanne (CH) / 09.04.2008
- *Gabriele Varani*
  "Structure-based design of new and potent inhibitors of HIV replication"
  University of Washington (US) / 09.05.2008
- *Bernice Lo*
  "The Role of Alveolar Epithelial Type II cells in T cell Tolerance: A Potential Role in Allergy and Inflammation"
  Duke University, Durham, North Carolina (US) / 03.05.2008
- *Federica Benvenuti*
  "Expression of Wiskott-Aldrich syndrome protein in dendritic cells regulates synapse formation and activation of naive CD8+ T cells"
  International Center for Genetic Engineering and Biotechnology Area Science Park, Trieste (IT) / 14.05.2008
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  "The role of miR-21 in lymphocyte biology and lymphoproliferative disease"
  "La Sapienza"University of Rome (IT) / 29.05.2008
- *Vincenzo Barnaba*
  "Cross-presentation, Immune-regulation & Immunopathology"
  "La Sapienza"University of Rome (IT) / 29.05.2008
- *Wolf-Dietrich Hardt*
  "Triggering of gut inflammation by Salmonella typhimurium: Dendritic cells, type III secretion and stochastic gene expression"
  Institute of Microbiology, ETH Zurich (CH) / 16.06.2008
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  Brigham and Women's Hospital, Harvard Medical School, Boston (US) / 18.06.2008
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  Anderson Cancer Center, Houston (US) / 18.07.2008
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  Heinrich-Pette-Institute for Experimental Virology and Immunology, Hamburg, (DE) / 29.07.2008
- *Mario Roederer*
  "Multicolor Flow Cytometry: Unravelling Immune Responses to Vaccine and Natural Infection"
  Vaccine Research Center, NIAID, NIH, Bethesda (US) / 31.07.2008

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  "Tumor-induced barriers to immune assault"
  Venetian Institute for Molecular Medicine, Padova (IT) / 30.10.2008
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  "Early events in B cells response to antigen"
  Cancer Research UK, London Research Institute (UK) / 03.11.2008
- *Marco Gattorno*
  "The Autoinflammatory diseases: from gene sto treatment"
  Giannini Gaslini Scientific Institute, Genoa (IT) / 21.11.2008
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  "Pediatric Systemic Lupus Erythematosus: a genetic approach"
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  Advanced Biotechnology Center, Genoa (IT) / 15.01.2009
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  WHO-IRTC, University of Lausanne, Epalinges (CH) / 19.01.2009
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  "Modulation of dendritic cell functions by prostaglandin E2"
  Biotechnology Institute Thurgau at the University of Konstanz, Kreuzlingen (CH) / 20.01.2009
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  "Temporal Specification of Blood Progenitors from Pluripotent Stem Cells"
  McEwen Center for Regenerative Medicine, University Health Network (UHN), Toronto (CA) / 03.02.2009
- *Ruth M Ruprecht*
  "A Novel Strategy for Immunofocusing"
  Dana-Farber Cancer Institute, Harvard Medical School, Boston (US) / 24.02.2009
- *Fulvio Reggiori*
  "Autophagosomes and other double-membrane vesicles. All the same?"
  Cell Microscopy Center and Institute of Biomembranes, University Medical Center (UMC), Utrecht (NL) / 27.02.2009
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  "Cortical demyelination occurs early in MS and sheds new light on lesion pathogenesis"
  Neuroinflammation Research Center, Cleveland Clinic, Cleveland (US) / 02.03.2009
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  "Chemokine/glycosaminoglycan ineractions: from structure do *in vivo* analysis"
  Institut Pasteur, Paris (FR) / 26.03.2009
- *Jens Stein*
  "Visualizing lymphocyte migration and activation *in vivo*"
  Theodor Kocher Institute, University of Bern (CH)/ 08.04.2009
- *William R Heath*
  "Antigen presentation, priming and tissue-resident T cell memory to HSV-1"
  University of Melbourne (AU) / 12.06.2009

SECTION 4

PEOPLE & FINANCES

INDEX SECTION 4 - PEOPLE & FINANCES

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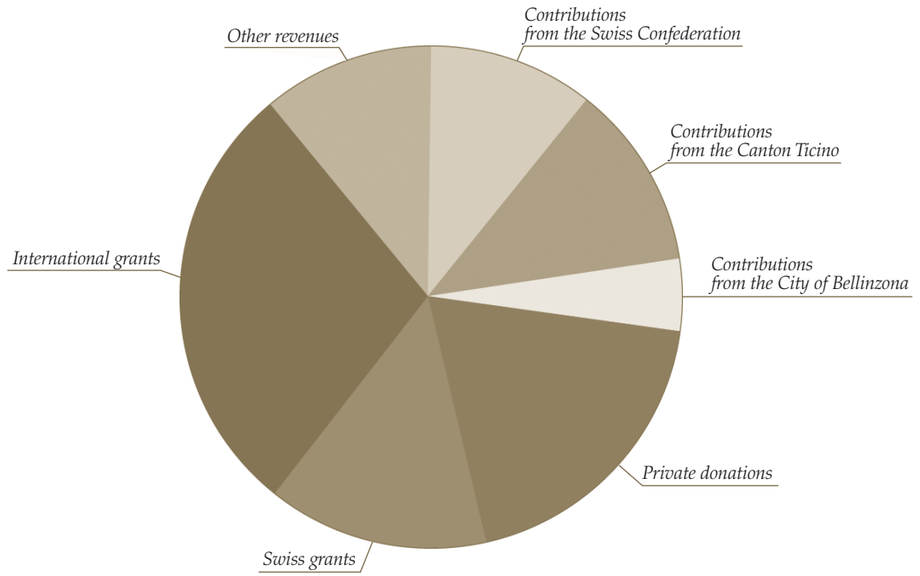
Financial Data 2007-2008

The year 2007 was very positive in terms of the financial consolidation of the institute and saw an important increase in research projects funded by international agencies.The overall costs of the institute increased from 11,3 million to 16 million, covered entirely by current revenue. From the point of view of investments, 2007 should be considered exceptional in that during the year new facilities were opened with building costs of 1.7 million and equipment costs of 2.5 million. Individual donors and friends of the institute covered these extra-costs. For the IRB, 2008 was a year of consolidation that saw a further development of the research activities in the laboratories. Overall costs were reduced by 1,5 milion CHF, though it is important to remember that 2007 costs included one-time investments covered by extraordinary donations. Research costs for 2008 were covered completely and increased from 6.2 to 6.8 million CHF.

Funding by source 2007-2008



Balance Sheet as 31 of December 2008 (in CHF)

ASSETS		**31.12.2008**	**31.12.2007**
1.	Liquidity	6'988'697	5'668'643
2.	Various Receivables	1'731'867	1'385'480
3.	Temporary Receivables	694'109	1'200'135
	Current Assets	***9'414'673***	***8'254'258***
4.	Participations	12'500	12'500
5.	Buildings	5'624'440	6'117'440
6.	Furnishing & Equipment	2'300'000	2'802'000
	Fixed Assets	***7'936'940***	***8'931'940***
	Total Assets	17'351'613	17'186'198

LIABILITIES		**31.12.2008**	**31.12.2007**
1.	Debt for Delivery and Services	562'654	1'117'969
2.	Accruals	523'553	341'051
3.	Funds for Research Projects	1'669'065	1'816'160
4.	Funds for Laboratories	1'655'808	1'559'005
5.	Various Funds	1'528'480	950'000
	Current Liabilities	***5'939'561***	***5'784'185***
6.	Long Term Loans	4'500'000	4'500'000
	Long Term Liabilities	***4'500'000***	***4'500'000***
7.	Capital Resources	6'902'013	6'806'793
8.	Annual Result	10'039	95'220
	Equity of the Foundation	***6'912'053***	***6'902'013***
	Total Liabilities	17'351'613	17'186'198

Profit and Loss Account for the year 2008 (in CHF)

COSTS		**2008**	**2007**
1.	*Personnel Costs*	6'573'830	5'819'323
2.	*Consumables*	2'155'474	2'203'889
3.	*Maintenance of Buildings and Equipment*	548'218	531'760
4.	*Investments*	861'582	2'547'655
5.	*Amortizations*	995'000	1'645'260
6.	*Rent and Related Costs*	1'271'104	1'117'212
7.	*Administrative Costs and Various*	945'145	951'743
8.	*Travel, Congresses and Guests*	396'457	360'620
9.	*Financial charges*	52'877	*76'193*
10.	*Various Costs for Research*	672'311	754'700
	Total Costs	14'471'999	16'008'355

REVENUE		**2008**	**2007**
1.	*Contributions from the Confederation*	1'334'000	*1'088'000*
2.	*Contrib. from the Canton Ticino*	2'000'000	*2'000'000*
3.	*Contrib. from the City of Bellinzona*	720'000	*665'000*
4.	*Contributions from the Helmut Horten Foundation*	1'500'000	*1'500'000*
5.	*Other Contributions*	1'527'198	*3'826'777*
6.	*Research Projects*	6'157'543	*5'974'220*
7.	*Other Revenue*	1'243'297	*1'049'578*
	Total Revenue	14'482'038	16'103'575
	ANNUAL RESULT	**10'039**	**95'220**

SECTION 5

PUBLICATIONS

INDEX SECTION 5 - PUBLICATIONS

Publications are numbered progressivly since the founding of the IRB in 2000

Peer Reviewed Publications
from July 2007

180. ***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, Dimitrov D S.
Proc Natl Acad Sci U S A. 2007;104:12123-12128.

181. ***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 MR, Nilsson M, Eriksson PS, Hagberg H, Luban J, Kroemer G, Blomgren K.
J Exp Med. 2007;204:1741-1748.

182. ***Substrate-specific requirements for UGT1-dependent release from calnexin.***
Soldà T, Galli C, Kaufman RJ, Molinari M.
Mol Cell. 2007;27:238-249.

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

184. ***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, Sallusto F.
Nat Immunol. 2007;8:942-949.

185. ***Membrane translocation of P-Rex1 is mediated by G protein betagamma subunits and phosphoinositide 3-kinase.***
Barber M A, Donald S, Thelen S, Anderson K E, Thelen M, Welch H C.
J Biol Chem. 2007;282:29967-29976.

186. ***In and out of the ER: protein folding, quality control, degradation, and related human diseases.***
Hebert D N, Molinari M.
Physiol Rev. 2007;87:1377-1408.

187. ***Identification of clonogenic common Flt3+M-CSFR+ plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow.***
Onai N, Obata-Onai A, Schmid M A, Ohteki T, Jarrossay D, Manz M G.
Nat Immunol. 2007;8:1207-1216.

188. ***CCL21 expression pattern of human secondary lymphoid organ stroma is conserved in inflammatory lesions with lymphoid neogenesis.***
Manzo A, Bugatti S, Caporali R, Prevo R, Jackson D G, Uguccioni M, Buckley C D, Montecucco C, Pitzalis C.
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189. ***Pillars article: Antigen-specific interaction between T and B cells. 1985.***
Lanzavecchia A.
J Immunol. 2007;179:7206-7208.

190. ***Human monoclonal antibodies by immortalization of memory B cells.***
Lanzavecchia A, Corti D, Sallusto F.
Curr Opin Biotechnol. 2007;18:523-528.

191. ***Macrophage tolerance: CD47-SIRP-alpha-mediated signals matter.***
Takizawa H, Manz M G.
Nat Immunol. 2007;8:1287-1289.

192. ***The strength of T cell stimulation determines IL-7 responsiveness, secondary expansion, and lineage commitment of primed human CD4+IL-7Rhi T cells.***
Lozza L, Rivino L, Guarda G, Jarrossay D, Rinaldi A, Bertoni F, Sallusto F, Lanzavecchia A, Geginat J.
Eur J Immunol. 2008;38:30-39.

193. ***Of Omenn and mice.***
Marrella V, Poliani P L, Sobacchi C, Grassi F, Villa A.
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194. *Cdk9/Cyclin T1 complex: a key player during the activation/differentiation process of normal lymphoid B cells.*
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196. *A role for microRNAs in the development of the immune system and in the pathogenesis of cancer.*
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Pipkin M E, Monticelli S.
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200. *A dual task for the Xbp1-responsive OS-9 variants in the mammalian endoplasmic reticulum: inhibiting secretion of misfolded protein conformers and enhancing their disposal.*
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IMPRESSUM

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