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 Stadues, 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.

In 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 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 is the establishment of a long term in vitro culture of human memory B cells of very high affinity. This culture is a very useful tool for the discovery of high affinity antibodies.

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. Marz, 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.

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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 is the establishment of a long term in vitro culture of human memory B cells of very high affinity. This culture is a very useful tool for the discovery of high affinity antibodies.
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
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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 Umeå 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 immunopathological 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 CD+ 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 Frasiotti, 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 βTCR 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 signalizing 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 vitro 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 accumulates 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 proteins 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.

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 T cell lineage and acquisition of the CD44+, double positive (DP) phenotype. In contrast, P2X7 was prominently expressed in DN cells upon productive rearrangement of βTCR and commitment to the γδ lineage. Stimulation with 1 mM ATP led to a cytosolic Ca2+ rise in a significant percentage of sorted CD4-8-2544 double negative 3 (DNS) thymocytes (in which productive TCR gene rearrangement determines lineage commitment), when Ca2+ was present in the extracellular medium. ATP triggered also a moderate cytosolic Ca2+ rise, due to IP3 mediated Ca2+ release from the ER stores when applied in the absence of extracellular Ca2+, thus confirming the presence of metabolotropic P2Y receptor(s). Using P2X subtypes preferring agonists we found that DN3 cells were activated following stimulation with BuATP, a P2X7 specific agonist and UDP-Glucose, a P2Y14 agonist. Therefore, the P2 receptor subtypes detected by RT-PCR were also functionally competent in DNS thymocytes. In peripheral T cells, we showed that P2X activation by ATP release 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 CD oligomerization, provided suppression of P2X expression during 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 CD 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 in co-culture of DN cells with the stromal cell line OP9 transfected with Notch ligand. Since ATP release from the ER stores when applied in the absence of extracellular Ca2+, thus confirming the presence of metabolotropic P2Y receptor(s). Using P2X subtype preferring agonists we found that DN3 cells were activated following stimulation with BuATP, a P2X7 specific agonist and UDP-Glucose, a P2Y14 agonist. Therefore, the P2 receptor subtypes detected by RT-PCR were also functionally competent in DNS thymocytes. In peripheral T cells, we showed that P2X activation by ATP release 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 CD 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 in co-culture of DN cells with the stromal cell line OP9 transfected with Notch ligand.
**ATP as an autocrine costimulus in T cell activation**

Ursula Schenk and Fabio Grassi

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 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 vitro 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: peroxidized ATP activates P2X receptors on the T cell in an autocrine fashion (4) and perhaps P2XR receptors on the APC in an paracrine fashion resulting in IL-1β processing and release (5).

![Schematic representation of T cell activation](attachment:diagram.png)

**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 immune complex deposition. Lupus-prone NZB/NZW F1 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 [1]. The molecular mechanisms responsible for increased responsiveness of effective/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 with 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. ATP induces autocrine purinergic hyperpolarization, we asked whether this might imply enhanced purinergic T cell costimulation contributing to SLE pathogenesis. Indeed mitochondrial Ca\(^{2+}\) uptake was enhanced in effector/memory T cells isolated from NZB/NZW F1 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 F1 mice with oxidized ATP (oATP) as a purinergic P2X receptor 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 F1 mice. These results suggest that effector/memory T cells in NZB/NZW F1 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 antagonist significantly ameliorated immunopathology in NZB/NZW F1 mice and thus might represent a therapeutic strategy to control SLE-associated glomerulonephritis. This project is done in collaboration with Elisabetta Taggarelli, 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 (oxATP) induces a type 1 helper T cell response. Analysis of major 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 oxATP, as opposed to the progressive upregulation of T-bet in untreated cultures. Furthermore, stimulation of sorted CD4+CD25high+ cells comprising natural Treg cells induced the expansion of Treg cells with higher expression levels of Foxp3 when oxATP was added to the culture medium. In T cells stimulated by anti-CD3 under Th17 skewing conditions (TGβ- and IL-6 conditioned medium) the absolute number of CD4+CD25high+ cells expressing Foxp3 was augmented by oxATP. The presence of oxATP 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+ naïve T cells toward the Treg lineage, as assessed by Foxp3 expression. However, Treg cells expanded in vitro oftentimes lose their suppressive phenotype and convert into potentially pathogenic Th17 cells. Addition of oxATP to culture medium prevented the conversion of sorted CD4+CD25high+ Treg cells to the Th17 lineage following stimulation. Contemporaneous injection of oxATP with CD4 naïve 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 native CD4+ cells together with Treg cells as healthy controls, suggesting that the presence of oxATP 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


We have shown that inhibition of partner P2X receptor signaling in activated T cells by oxidized ATP (oxATP) induces a type 1 helper T cell response. Analysis of major 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 oxATP, as opposed to the progressive upregulation of T-bet in untreated cultures. Furthermore, stimulation of sorted CD4+CD25high+ cells comprising natural Treg cells induced the expansion of Treg cells with higher expression levels of Foxp3 when oxATP was added to the culture medium. In T cells stimulated by anti-CD3 under Th17 skewing conditions (TGβ- and IL-6 conditioned medium) the absolute number of CD4+CD25high+ cells expressing Foxp3 was augmented by oxATP. The presence of oxATP 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+ naïve T cells toward the Treg lineage, as assessed by Foxp3 expression. However, Treg cells expanded in vitro often lose their suppressive phenotype and convert into potentially pathogenic Th17 cells. Addition of oxATP to culture medium prevented the conversion of sorted CD4+CD25high+ Treg cells to the Th17 lineage following stimulation. Contemporaneous injection of oxATP with CD4 naïve 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 native CD4+ cells together with Treg cells as healthy controls, suggesting that the presence of oxATP 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 recombinease activity associated with generalized erythrodema, alopecia, lymphadenopathy, hepatosplenomegaly, and intractable diarrhea. Oligosporal 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 (Treg). 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 junction 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, cytokerin P490 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-2-/- mice by injection of CD3e specific antibodies, a treatment that impairs thymocyte/epithelial cross-talk mediated by p-TCR expression, which is missing in RAG-2-/- mice. One week after anti-CD3e administration, mice were transplanted with EL4 lymph node progenitors from Rag2-/- mice. Eight weeks after transplantation, they were evaluated to analyze T cell differentiation and immunopathology. In contrast to Rag2-/- mice and fetal liver chimeras in RAG-2-/- mice, fetal liver chimeras in RAG-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).

Lymphopoeisis 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
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<sup>-</sup>CD44<sup>+</sup>62L<sup>-</sup>effector/memory T cells in the absence or presence of tissue inflammation. The increase in peripheral CD4<sup>-</sup>CD44<sup>+</sup>62L<sup>-</sup> 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 = 0.034) mice, the interaction term was not significant (P = 0.98) 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 ef-
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  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
  FP6 - LSHG-CT-2005-005203 / 2005-2010

- Oncosuisse
  Synergy between oncogenic Notch and pre-T cell receptor (pre-TCR) signaling microdomains in leukemogenesis
  01/03 / 2007-2009

- Tecnico Foundation for Cancer Research
  (Fondazione Tecnico per la Ricerca sul Cancro)
  Synergy between oncogenic Notch and P2 receptors in leukemogenesis
  2006-2008

- Tecnico Foundation for Transfusion and Transplantation Research
  (Fondazione per la 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

Publications

- A hypomorphic R229Q Rag2 mouse mutant recapitulates human Omenn syndrome.

- Of Omenn and mice.
  Marello V, Poliani P L, Sobacchi C, Grassi F, Villa A.

- Recombinase-deficient T cell development by selective accumulation of CD3 into lipid rafts.
  Ferrera D, Pescud M, Prudente S, Grassi F.

- Purinergic control of T cell activation by ATP released through pannexin-1 hemichannels.

Collaborations

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- Eugenio Scanziani
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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 (CR) / 29.01.2008

- Purino 2008
  Copenhagen (DK) / 30.06.2008

- ThymoIS
  San Juan (PR) / 11.11.2008

- University of Florence
  Seminar “Purinergic signaling in T cell physiology”
  Florence (IT) / 18.11.2008
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 Bernasceni, 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

Antonello 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 endothelial 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 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 gH, gL 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 secreting plasma cell lines from PBMC of HIV-1 infected donors. We immortalized IgG secreting plasma cell lines from PBMC of HIV-1 infected donors. To achieve this, we undertook the task of isolating human monoclonal antibodies from non clade B HIV-1 infected donors. IgG secreting plasma cell lines from PBMC of HIV-1 infected donors were seeded as single cells on MSC monolayers and IgG production was monitored using a combination of R8 8 and IL-2. In these conditions 0-0% 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.

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

David Jarrossay, Davide Corti, Annalisa Macagno, Giovanna 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 hypothesised 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-
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 (H1, H, 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.

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 (Dsg3)and Dsg4. 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 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 autoantibodies and vaccine-specific B cells during the B cell depletion and reconstitution phase.

This project is done in collaboration with Gaiaauma Zambros and Giovanni Di Zenzo, INR, 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 P27 and P27A 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 potentially 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 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 T cell stimulation and cytokine production that are not beneficial for the host since they are not targeted to the pathogen.

On the cellular basis of IgE memory

Giosiana Codoni, Giulia Di Laia, Annalisa Macagn 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 hematopoietic 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
- Siorus National Science Foundation
  Role of receptors in dendritic cell and lymphocyte activation
  310260-12678 / 2006-2009
- Siorus National Science Foundation
  Role of receptors in dendritic cell and lymphocyte activation
  31003A-126072 / 2009-2012
- Human Frontier Science Program
  Human immune memory and vaccine responses: high throughput analyses of antibody repertoire and function
  RCP-2007 / 2007-2010
- European Union
  HUMALMAP: Human monoclonal antibodies as cells for malaria research and therapy
  FP6-LHSP-CT-2006-03880 / 2007-2008
- European Union
  IMEMS: Identification of mechanisms correlating with susceptibility for avian influenza
  FP7-201169 / 2008-2012
- European Union
  PEMPHIGUS: From autoimmunity to disease
- European Union
  MUCO-2112-0031 / 2006-2009
- National Institute of Health (US)
  Vaccine-induced protective cross-neutralization of HIV-1
  867 / 2006-2011
- Human Frontier Science Program
  Human immune memory and vaccine responses: high throughput analyses of antibody repertoire and function
  RCP-2007 / 2007-2010
- European Union
  DC-TBERA: Dendritic cells for immunotherapy

Collaborations
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  IRL, Rome (IT)
- Fabio Grassi
  NICHD, Bethesda (US)
- Silvia Monticelli
  University College, London (UK)
- Cdk9/Cyclin T1 complex: a key player during lymphocyte activation
- Human Th17 cells in infection and autoimmunity
- Prostaglandin E2 enhances Th17 responses via modulation of IL-17 and IFN-gamma production by memory CD4+ T cells
- CD4+ memory T cells migrate in a CD62P-dependent fashion into reactive lymph nodes and license dendritic cells for T cell priming
- Structural basis for potent cross-neutralizing human monoclonal antibody protection against lethal human and nonhuman primate respiratory coronovirus challenge
- Lineage commitment of primed human CD4+Th17 T cells

Book chapters

Lectures and Seminars
• Cambridge Immunology Forum Cambridge (UK) / 27.09.07
• PPTDC Discovery & Immunology Cores Meeting New York, NY (US) / 05.10.07
• XVth International Immunotherapy Symposium Series Meeting New York, 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, Immuno-pathology and Immuno-regulation: 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
• LAIV – R&D Retreat New York, NY (US) / 13.03.08
• Biogenesis - Tregs and Th17 Cells in Autoimmunity Washington, DC (US) / 26.03.08
• International Materniti Foundation – Respiratory Allergy & COPD from Inmate Immunity to Treatment Geneva (IT) / 11.04.08
• LAIV – NAC Neutralising 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 SICRA Rome (IT) / 14.06.08
• B Cell Conference Oxford (UK) / 20.06.08
• 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) / 19.08.2008
• The Scripps Research Institute Seminar "Isolation and characterization of human monoclonal antibodies" Jupiter, FL (US) / 12.10.2008
• Symposium in Honor of Rafi’s 60th Birthday Atlanta, GA (US) / 13-16.11.2008
• Malfan Vaccines Consultative Meeting Montreux (FR) / 22.11.2008
• Midwinter Conference of Immunologists Dunn-Campbell Lecture Aalstom, CA (US) / 24.01.2009
• USGER Annual Meeting STSR 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
• ELJBO-PAdNet Consortium Meeting Rome (IT) / 04.04.2009
• Yale University Janeway Memorial Lecture "Dissecting the immune response to pathogens" New Haven, CT (US) / 09.04.2009
Markus G. Manz received his degree as M.D. in 1995 at the Eberhard-Karls-University Medical School in Tübingen, 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 Tübingen 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 hematology/oncology training and board qualification at the University of Tübingen, 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 2005, 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 hematopoietic system, a system with very high cellular turnover. 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 40 years for the treatment of malignant hematologic disease or immunodeficiencies. In addition, hematopoetic 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 hematopoietic differentiation in physiologic and inflammatory 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.

Team
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Members: Sekhar Boddupalli, PhD student – Chiara Bonvetti, PhD student – Daniela Bossi, PhD – Dior Kingston, PhD student – Rouven Mueller, MD – Michael Schmid, PhD student – Hitoshi Takizawa, PhD – Patrick Ziegler, PhD
Human hematopoietic stem cells (HSCs) are crucial for maintaining the hematopoietic system throughout life. The goal of our research is to understand the biology and dynamics of HSCs, which will help in developing therapies for diseases such as cancer and immunodeficiency.

**Research Projects**

1. **Hematopoietic stem cell population dynamics**

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

   Because ethical and practical restrictions limit in vitro studies in humans, small animal models that faithfully resemble human physiology and pathology in vivo settings are valuable for predictive in vivo preclinical testing. Multiple examples of 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-/- mice leads to some 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 to vaccines, and the dynamics of human hematopoietic system specific immune responses, 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-/- and other immunodeficient mice, further improvement for faithful resemblance of the human hematopoietic system in this small animal model is warranted. A collaborative, Gates Foundation Grant Challenges in Global Health Program supported project we are thus re-placing 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).

   **Human specific viral infection in Human-Hematolymphoid System Mice.**

   Hitoshi Takizawa, Sekhar Reddappa, 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 ablative 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) 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.

2. **Dendritic cell homeostasis**

   Dior Kingston, Sekhar Reddappa, 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 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) nonlym- phoid tissue migratory and lymphoid tissue resident DCs and (2) plasmacytoid DCs (pDCs), also called natural interferon-producing cells, (IPC). 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. erythrophagocysis or thrombopoiesis) 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 Flt3 in 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 (Isl1 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.
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-CSF 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-CSF 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 reception of the livers 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.

**Hematopoiesis supporting mesenchymal stromal cells**

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

Adult mammalian hematopoiesis, 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) 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−/− mice. Upper panel: Apopt HE stain demonstrating multiple mitosis, lower panel shows FACS analysis using anti human CD34 and CD45 antibodies*
RESEARCH PROJECTS

- The STAT5 on dendritic cell development.

- CD4+ cord blood-transplanted Rag2-/-gamm(a)-/- mice as a model for Epstein-Barr virus infection.

- Human T cell development and HIV infection in human hematopoietic system mice.

- Transient depletion of RUNX1/RUNX1T1 by RNA interference delays tumor formation in vivo.

- Dendritic cell hematopoiesis.

- FBSL, DXA, and N-Exo: tono contra GVHD?

- The concerted action of GM-CSF and Flt-3 ligand on in vitro dendritic cell hematopoiesis.

- Humanized mice for modeling human infectious disease: challenges, progress, and outlook.

- Potent inhibition of HIV-1 by TRIM5-α cyclophilin fusion proteins engineered from human components.

- Book chapters

- Lectures and Seminars
  - Keystone Symposium on Immunological Intervention in Human Disease
  - Heidelberg Akademie der Wissenschaften, University of Heidelberg
  - Hematopoietic Lecture "Animal models in stem cell research" in Heidelberg (DE) / 02.02.2007.
  - Heinrich-Petits-Institut Seminar "Human immune system Rag2-/-gamm(a)-/- mice: New options to study human lymphopoiesis in vitro" in Hamburg (DE) / 02.03.2007.
  - DC Event: DC-THERA Graduate School Cologne (CH) / 30.03.2007.
  - The Swiss Society for Allergy and Immunology Annual Meeting and Spring Meeting SGIV-DDS
  - Basel (CH) / 20.04.2007.
  - 21st European Immunogenetics and Histoocompatibility Conference, European Federation for Immunogenetics
  - Barcelona (ES) / 07.05.2007.
  - Annual retreat of the infection Biology Program at Karolinska Institute
  - Lecture "Human hematopoetic lymphoid system mice" in 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" in Zurich (CH) / 23.10.2007.
  - University of Bern Immunology Seminar series "Dendritic cell differentiation and hematopoiesis" in Bern (CH) / 31.10.2007.
  - The 37th Annual Meeting of the Japanese Society for Immunology
  - University of Geneva Biology Seminar series "Dendritic cell development and homeostasis"
  - The Immunology Institute Mount Sinai School of Medicine Seminar "Human Hemato-Lymphoid System Rag2gamm(a)-/- mice" in New York, NY / 03.04.2008.
  - University of Basel DBM-Schwerpunktseminar "From hematopoetic stem to immune system cells" in Basel (CH) / 15.02.2008.
  - University of Freiburg Hematology Lecture "Humar Hämatopoese im Mäus-Modell: Diagnostische und thera-
  - peutische Perspektiven" in Freiburg (DE) / 22.02.2008.
  - Weizmann Institute of Science Seminar "hemato-lymphoid system differentiation, homeostasis, and neoplasia" in Rehovot (IL) / 27.03.2008.
  - European Institute of Oncology Seminar "Human immune system mice as preclinical testing Models" in 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 "hematopoetic stem cell dynamics" in Lichtenstein (CH) / 18.07.2008.
  - 10th International Symposium on Dendritic Cells Kobe (JP) / 01.10.2008.
  - IgE organized by Oncology Institute of Southern Switzerland Bellinzona (CH) / 16.10.2008.
• Life Science College Peking University
  Seminar “Human-hematopoietic system mice”
  Beijing (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

• Heidelberg University
  Hämatologikum Seminar “Divisional dynamics in hematopoietic stem cells”
  Munich (DE) / 27.11.2008

• Yale University
  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

• IL 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 –Th17R and the pathogenesis of hematic 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 vitro 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 intracellular or extracellular destination. Folding-defective polypeptides are exported across the ER membrane into the cytosol and destroyed (see 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, Undergraduate student – Verena Calanca, Technician – Tito Calì, PhD student – Christian Caprara, Undergraduate 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 intraluminal level of OS9.1 and OS9.2 inversely correlates with the fraction of a folding-defective glycoprotein, the Null_anti-1-anti-2 variant (NHK) of α1-antitrypsin, that escapes retention-based ER quality control. OS9 up-regulation does not affect NHK disposal, but reduction of the intraluminal 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).

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 glycosylation system. Under normal growth conditions, the intraluminal 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 the bulk ER turnover. 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.

The fate of newly synthesized glycoproteins in the ER lumen

Nascent chains enter the ER lumen through the Sec61 complex. They are core glycosylated by the oligosaccharyltransferase (OST). The two terminal glucose residues are rapidly trimmed by sequential action of the glucosidase I and II (step 1). Mono-glucosylated N-glycans mediate initial association of folding polypeptides with the ER lectin chaperones calnexin and/or calreticulin (Cnx/Crt) and exposure to the glycoprotein-dependent retro-translocation machinery for proteasome-mediated degradation (step 4b).

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Molecular characterization of ERAD tuning
Sim Bianchi and Maurizio Molinari

We define as ERAD tuning the posttranslational 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 intraluminal 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 Fabio Reggiori, University of Utrecht (NL).

Consequences of N-glycan deletion and posttranslational inhibition on secretion of active BACE
Omar Vanoni and Maurizio Molinari

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 binding to a di-glucosylated high-mannose N-glycan sequence. Glc, Man, GlcNAc. 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 Malce in mammalian cells as well as cell lines characterized by low content in endogenous Malec, 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, University of Lausanne for analysis. Tysonic fragments will be separated by nan-HPLC followed by tandem MS. Fragmentation spectra for trypsin fragments 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 Ten Feizi, Imperial College London (UK) and Markus Aebi, ETH-Zurich (CH).

Characterization of Malec in a novel ER-resident lectin
Riccardo Bernassoni, Verena Calanca and Maurizio Molinari

This project is concerned with a newly discovered ER-resident carbohydrate–binding protein named Malec. First detected in Xenopus laevis, Malec 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, Man, GlcNAc. 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 Malec in mammalian cells is therefore of great interest. We prepared vectors for expression of Malec in mammalian cells as well as cell lines characterized by low content in endogenous Malec (obtained by stable RNA interference). Consequences of Malec 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, University of Lausanne for analysis. Tysonic fragments will be separated by nano-HPLC followed by tandem MS. Fragmentation spectra for trypsin fragments 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 $\beta$-peptide

Siro Bianchi and Maurizio Molinari

Sequential clearances 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 Aβ thus substantially interfering with beta- and gamma-secretase cleavage and Aβ production (Pagnetti et al. 2010). When added extracellularly, they associate with surface exposed Aβ 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 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 Alzheimer’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 inter- and inter-molecular disulfide bonds and cis/trans isomeration of prolyl bonds are rate-limiting steps for folding of nascent polypeptides. A role of oxido-reductases in polypeptide maturation is well documented, while a role of peptidyl prolyl cis/trans isomerasers (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 PPIs (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 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 Alzheimer’s disease (ongoing).

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

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 the gp78-regulated ERAD pathway.

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, Japan (JP)


drugs of choice

Antibiotics/Antivirals

Antihistamines

Antineoplastics

Antileukemics

Anticonvulsants

Antipsychotics

Antidepressants

Antihypertensives

Antianxiety agents

Antiallergics

Antidiabetics
DEDEM1, DEDEM2 and DEDEM3 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, DEDEM1 would act as a receptor for misfolded polypeptides to be degraded, as a mannosidase, DEDEM1 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 DEDEM1 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 DEDEM1 with purified components in test tubes. The aim of this project is to determine if mammalian DEDEM1 and DEDEM2, a protein recently characterized in our lab (Olivari et al. 2008) 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
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- Toshihiro Nakajima
- Ten Feizi
- Manfredo Quadroni
- Fulvio Reggiori
- Swiss Foundation for Aging Research
- San Salvatore Foundation

Plasticity and Repair

- NCCR of Competence in Research on Neural Degenerative Diseases

Publications


Collaborations

- Markus Aebi
  Swiss Federal Institute of Technology (ETH), Lausanne (CH)
- Paola Paganiotti
  Novartis Pharma, Basel (CH)
- Patrick Aebischer
  Ecole Polytechnique Federale de Lausanne (EPFL), Lausanne (CH)
- Jeremy Luban
  University of Geneva (CH)
- Paolo Paganiotti
- Novartis Pharma, Basel (CH)
- Patrick Aebischer
  Ecole Polytechnique Federale de Lausanne (EPFL), Lausanne (CH)

Book chapters

- Annual Meeting of the Union of the Swiss Societies for Experimental Biology (USGBE) Interlaken (CH) / 29.01.2009
- Forum on Biochemistry of Signal Transduction
  Milan (IT) / 24.02.2009
- 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) / 15.05.2009
- 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”
  Saxton River, VT (US) / 07.06.2009
- Cellular and Molecular Responses to Stress
  Milan (IT) / 15.06.2009

Lectures and Seminars

- Basic Virology Course, Institute 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, Institute 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
  Villa del Mare (CS) / 08.01.2009
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

Group Leader: Silvia Monticelli, PhD - silvia.monticelli@irb.unisi.ch

Members: Lorenzo Dehò, PhD student – Ramon J Mayoral, PhD student – Nicole Rusca, PhD student – Fabio Grassi – Antonio Lanzavecchia – Markus G Manz – Maurizio Molinari – Federica Sallusto – Marcus Thelen – Mariagrazia Uguccioni – Luca Varani
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/NF-κB 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 p27kip1. 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 vitro role in MC differentiation and functions by taking advantage of mouse models lacking MCs.

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-146 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 Th 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 Th 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 (HMCs) 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.

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**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.

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**Collaborations**

- Erik van Nimwegen  
  Biozentrum, University of Basel (CH)

- Anjana Rao  
  Immune Disease Institute and Harvard Medical School, Boston (US)

- Anton Enright  
  European Bioinformatics Institute, EMBL, Cambridge (UK)

**Publications**

- A role for microRNAs in the development of the immune system and in the pathogenesis of cancer.  
  Kanellopoulou C, Monticelli S.  

- Genomics and the immune system.  
  Pipkin M E, Monticelli S.  

- MicroRNA-221-222 regulate the cell cycle in mast cells.  
  Mayoral R J, Pipkin M E, Fleckov M, van Nimwegen E, Rao A, Monticelli S.  

**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  
  Brussels (BE) / 31.01.2009

- Keystone Symposium “MicroRNA and Cancer”  
  Keystone, CO (US) / 10.06.2009

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Details from July 2007 until June 2009
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<sub>CM</sub>) express homing receptors for lymph nodes and have no or low level effector function. In contrast effector memory T cells (T<sub>EM</sub>) lack lymph node receptors and have immediate effector function. We are further investigating the heterogeneity of memory T cell subsets and the role these subjects play in different physiological and pathological conditions.

Team

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Members: Martina Beltramello, PhD – Dirk Baumjohann, PhD student – Tess Brodie, Undergraduate student – Nina Chevalier, MD – Thomas Duhren, PhD – Rebekka Geiger, PhD student – Alfonso Martín-Fontecha, PhD – Luana Perlini, Technician – Andrea Reboldi, PhD student – Francesca Ronchi, PhD student – Christina Zielinski, MD
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 help upon boosting immunization. Although immunoglobulin class switch recombination, initiation of germinal center reaction, and differentiation to antibody-secreting short-lived plasma blasts were normal in reconstituted CD3ε⁻/⁻ 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.

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 remains 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 CNS and suggest that the CCR6–CCL20 axis in the choroid plexus controls immune surveillance of the CNS.

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 naïve 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-1α, 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 naïve T cells with dendritic cells and CD3 antibodies under standard Th1, Th2 and Th17-polarizing conditions. We found that naïve 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 naïve 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. Caspase-1-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 NCF-1-dependent fashion. These findings delineate a novel subset of human CD4+ effector T cells dedicated to skin physiology.

**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 constraints 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 Epstein-Barr virus, were detected at frequencies ranging from 5 to 170 cells per 10^6 cells. These cells showed a broad range of functional avidities over 100fold 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 homogeneously 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.

**Schematic representation of the T cell library method**

a) Library generation

- KLH, Anthrax, primary antigens
- Thyroglobulin (small antigens)
- TCR, CD4+, PBMC (large antigens)

b) Screening

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.

**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 Martin-Fuentes, Dirk Baumjohann, Greta Guarda, Andrea Reboli 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 (TEM), but not naive or central memory T cells (TCM), constitutively expressed CD40L at levels sufficient to induce DC maturation in vitro and in vivo in the absence of antigenic stimulation. TCM TEM cells were excluded from resting lymphoid 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+ TEM 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+ TEM migration into reactive lymph nodes, inhibited DC maturation, T cell priming and induction of EAE. These results show that TEM cells can behave as endogenous adjuvants and suggest a mechanistic link between lymphocyte traffic in lymph nodes and induction of autoimmunity.
Analysis of the human antibody response to Dengue virus and production of a broadly neutralizing cocktail of human monoclonal antibodies lacking infection enhancing activity

Marina Beltrame, Antonio Lanzavecchia and Federica Sallusto

Dengue (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 pM. These antibodies were broadly cross-reactive with the four DENV serotypes and neutralized weakly or failed to neutralize infection of susceptible targets, while potentially enhancing infection of cells expressing Fcγ 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 immunemediated 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 differentiation of interleukin 17-producing T cells. We found that Th17 cells produce immuno-suppressive 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 downregulation 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.

Intrathoracic trafficking of natural killer cells is redirected by chemokines upon inflammation.


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


Phenotype and function of human T lymphocyte subsets: consensus and issues.


Antigen fingerprinting of H5N1 avian influenza using conserved surface and monoclonal antibodies reveals potential vaccine and diagnostic targets.


Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells.


Human B cell memory.


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


Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells.


Human T cell memory.


Cholinergic control of the human memory B-cell repertoire following infection and vaccination.


C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE.


Human B cell memory.


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


Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells.


Book chapters


Lectures and Seminars

11th Annual Meeting of SFCI Tokyo (JP) / 11.07.2007

Annual Congress of the “Deutschen, Österreichischen und Schweizerischen Gesellschaft für Hämatologie und Onkologie” Basel (CH) / 05.10.2007

XXIV National Congress of the Italian society of Rheumatology Verona (IT) / 17.10.2007

Annual Meeting of the Caucasian 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 (AUS) / 02.12.2007

MASIR 2008 La Plagru (FR) / 30.01.2008

Ringberg Colloquium on “Determinism and plastiocity of T lymphocytes” Rotthach-Egern (DE) / 10.02.2008


Symposium Immunotherapy SFB 685 Taubingen (DE) / 08.03.2008

BioSymposia “Tregs and TH17 Cells in Autimun” 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 “Lymphocyte differentiation and regulation in disease” London (UK) / 12.09.2008

22nd European Conference & Dendritic Cell Society Annual Meeting Brocua (IT) / 18.09.2008


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 Inflammation and Disease Florence (IT) / 04.12.2008

Keynote Symposium “Mobilizing Cellular Immunity for Cancer Therapy” Snowbird (US) / 31.05.2009

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

Keynote Symposium “TH17 Cells in Health and Disease” Vancouver (CA) / 05.02.2009

Keynote Symposium “Immunological Memory and Host Defense” Keystone, CO (US) / 08.02.2009

Accademia Medica di Rome Lecture “Migracine e funzione effettore dei linfociti T nelle patologie autoimmuni” Rome (IT) / 05.03.2009

World Immune Regulation Meeting-III Davos (CH) / 23.03.2009

University of Genoa Seminar “Lymphocyte trafficking in immunity and autoimmunity” Genoa (IT) / 22.05.2009

RESEARCH PROJECTS
• 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-pathogen interaction”
  Helsingør (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 cytokine- 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 Thodor-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 1998 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

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**RESEARCH PROJECTS**

**Novel CXCR4-activated signal transduction pathways**

Tiziana Apuzzo and Marcus Thelen

Chemokine receptors belong to the rhodopsin-like subfamily of 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 hematopoiesis. 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-proteins, but has the ability to trigger the prolonged activation of intracellular signal transduction. Nevertheless, the presence of the chemokine receptors on a cell surface does not correlate with its activity profile. Thus, it is conceivable that CXCR4 in addition to G-protein 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 cytoskeletal proteins. Among the proteins we identified potential scaffolding molecules and proteins which could stand for a direct link of CXCR4 with NfNBF-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 Camerini 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 fluorochrome 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 trafficking of fluorescent 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 localization of the protein can be monitored and characterized. It is therefore not surprising that the functional role of chemokine receptors is 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 insights 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 chemotactic agent 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 chemotractants. The small concentration difference of the chemotractant along the polarization axis is probably insufficient to stimulate different receptor activities at the front and the rear. To explain the functional morphology, several mechanisms for cell polarization have been
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-protein coupled seven transmembrane containing receptors (GPCRs). Recent reports tagged with fluorescent probes were described to observe the activation of GPCRs by fluorescence energy transfer (FRET). We use alpha adrenergic receptors tagged with GFP 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 chemotactic 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 chemotactic 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 analyses 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-protein-mediated signaling poses the question if the receptor associates with an other 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.

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 GTases act as switches and are either on in their GTP bound form or off when loaded with GDP. Activation of the GTases is catalyzed by specific GTP exchange factors (GEF). The phosphatidylinositol 3,4,5-trisphosphate (PIP3)-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 PIP3 and by the βγ-subunits of heterotrimeric G-proteins, which are released upon activation of GPCRs. Consistent with the activation by these effectors and their cellular localizations, 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 of 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 immunostainchemistry. 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.

P-Rex1 expression in human esophagus epithelium. Upper panel: normal tissue. P-Rex1 is scarcely expressed in the epithelium. The arrowhead points to the endoderm of a blood islet 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
  3104A0-112341 / 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 autoimmune and chronic inflammation
• European Union
  MAIN: targeting cell migration in chronic inflammation
• European Union, Marie Curie Actions
  INTERGRAMM: International Graduate Program in Molecular Medicine
  2006 / 2006–2009
• Roche Research Foundation
  Membrane translocation of P-Rex1 is mediated by G protein betagamma subunits and phosphoinositide 3-kinase.
  CXCR7, CXCR4 and CXCL12: an eccentric trio?
  How chemokines invite leukocytes to dance.
  A cross-talk 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.

Lectures and Seminars
• University of Brescia
  Seminar “RDC1/CXCR7 a novel potential chemokine receptor”
  Brescia (IT) / 04.04.2008
  Meeting on Hormones and Cell Regulation
  – GPCR complexes and GPCR complexity
  Meet St. Odle (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

Collaborations
• Peter Gierschick
  University of Ulm (DE)
• John Hartwig
  Brigham and Women Hospital, Harvard Medical School, Boston (US)

Publications
• Membrane translocation of P-Rex1 is mediated by G protein betagamma subunits and phosphoinositide 3-kinase.
• CXCR7, CXCR4 and CXCL12: an eccentric trio?
• How chemokines invite leukocytes to dance.
• A cross-talk 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.
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 1995 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.

Mariagrazia Uguccioni
**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  
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**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

**RESEARCH PROJECTS**

**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.

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**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 synergise 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 synergise 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.

**Chemokines in chronic inflammation and autoimmunity**

Denise Bottinelli, Milena Schmidt 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, 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 Sallustro, IRR, Bellinzona (CH) and Marco Biondi, San Raffaele Institute, Milan (IT).
**Chemokines in tumors**

Daniel Yovetz, Maria Gabriela Danelon and Mariagrazia Ugucioni

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 chemotractants, 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 (CXCL12), 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 CXCR3, 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 CXCL12 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 Ugucioni

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
3100A0-118084/1 / 2008-2011

European Union
MAD: Targeting cell migration in chronic inflammation

European Union
TIP-VAC: Explaining and improving efficacy of targeted immunodeficiency virus-like particles vaccines against AIDS

European Union
DISC-VAC: Development of a dendritic cell-targeted vaccine against AIDS
FP6 – LSHP-CT-2005-018668 / 2005-2010

European Union
INNO-CHIM: Innovative chemokine-based therapeutic strategies for autoimmunity and chronic inflammation
FP6 – LSHP-CT-2005-018367 / 2005-2010

San Salvatore Foundation
Chemokine expression in extranodal lymphomas
2006-2010

Max Clefflate Foundation
Student Fellowship to Daniel Venetz
Molecular and biological mechanisms of cell trafficking in lymphoid organs. Involvement of chemokines in host homeostasis and tumour growth 2006-2009

European Union
 Marie-Curie Intra-European Fellowship to Valentina Cecchinato
MD-THIV: Migration and Differentiation of Th17 in HIV/SHIV infection
FP7 - PEPF-225280 / 2009-2011

Collaborations
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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)
• Marco Paroncini
Università Libre De Bruxelles (BE)
• Constantino Pitzalis
William Harvey Research Institute, University of London (UK)
• Maurilio Ponzoni and Claudio Doglioni
San Raffaele Institute, Milan (IT)
• Christiane Stahl-Henning
German Plasma Center, Goettingen (DE)
• Ralph Steinman
Rockefeller University, New York, NY (US)
• William Vorni
University of Bologna (IT)

Visiting Scientists
• Antonio Marin
William Harvey Research Institute, University of London (UK)
• Eleonora D’Introno
University of Bologna (IT)

Publications
• CCL21 expression pattern of human secondary lymphoid organ stroma is conserved in inflammatory lesions with lymphoid neogenesis.
• Mature antigen-experienced T helper cells synthesize and secrete the B cell chemokine CXCL13 in the inflammatory environment of the rheumatoid joint.
• Identification of CXCL13 as a new marker for follicular dendritic cell sarcoma.
• Synergy-inducing chemokines enhance CCR2 ligand activities on monocytes.
• Synthetic double-stranded RNAs are adjuvants for the induction of Th1 helper T and Th2 humoral immune responses to human papillomavirus in rhesus macaques.

Book chapters

Lectures and Seminars
• The Italian Society for Clinical Immunology and Allergy (SIRCA) Annual Meeting
Trieste (IT) / 06-07-2007
• 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 leukocyte trafficking”
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
Prague (CZ) / 14.06.2008
• 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 “Chemokines as potential therapeutic targets”
Bologna (IT) / 24.06.2009
• Academy of Science, University of Bologna
Lecture “Chemokines in the immunopathogenesis of chronic inflammation and lymphomas”
Bologna (IT) / 24.06.2009
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 computational demand. 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 previously infected vaccine could enhance subsequent infection by a different serotype and lead to Dengue Hemorrhagic Fever (DHF), a lethal form of the disease. 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 computational 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 AEs bound to domain III using an aptly modified version of the program “Rosetta-docking”, using the available experimental 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.
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)
  Maruno (CH)
• Luigi Calzolai
  European Union Joint Research Center, Ispra (IT)
• NMR center MRC-NIMR
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• Michele Parrinello
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Visiting Scientists
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Lectures and Seminars
• MRC-NIMR
  Structural Biology Seminar series “Rapid detection and characterisation 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 characterisation of TCR/pMHC interactions”
  Chatham (UK) / 20.04.2008
• Università degli Studi dell’Insubria
  Seminar “Rapid detection and characterisation of TCR/pMHC interactions”
  Busto Arsizio (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
Proteins expressed in the endoplasmic reticulum (ER) are covalently modified by cotranslational addition of pre-assembled core glycans (glucose9-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 glyco-polypeptides from the calnexin chaperone system, by accelerating de-mannosylation of terminally misfolded glycoproteins and by inhibiting formation of covalent aggregates. The intraluminal 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 intraluminal 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 glycoproteptides. 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.
Hematopoiesis during T cell mediated inflammation

Anna Casati

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 homoeostasis between cells of different lineages or increased demand of particular subsets 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/erythroid 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-/-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 pre-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 inflam-
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 R88 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 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.
Katrin Kuscher
Synergy-inducing chemokines enhance CCR2 ligand activity on monocytes

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. Moreover, 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.

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

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 expanded and differentiated 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 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.
Debora Pinna

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

Supervisor: Antonio Lanzavecchia // Co-referees: Edizio Gussi
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 to characterize the heterosubtypic antibody response to the classical Influenza vaccine. Here we report that the classical Influenza vaccine triggers the expansion and differentiation of human memory B cells with IgM and IgG specificities against a broad range of antigenic determinants. These antibodies were also protective in an in vivo setting.

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 β-site APP-cleaving enzyme 1 (BACE1) 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 BACE1 variants revealed a direct correlation between the number of N-glycans displayed on the nascent polypeptide chains and folding efficiency in the calcium chaperone system, folding rate and secretion. Addition of 1 to 3 N-glycans progressively enhanced the dissociation rate from BiP and reduced the propensity of newly synthesized BACE1s to enter aberrant soluble and insoluble aggregates. Finally, inhibition of the proteasome increased the yield of active BACE1s secreted from cells. This suggested that a fraction of BACE1s 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 in the regulation of the folding and degradation machineries competing during protein biogenesis. UGT1 is a glycosyltransferase enzyme that can attain the native structure. Protein-bound oligosaccharides determine the fate of newly synthesized polypeptides and how folding and degradation machineries compete during protein biogenesis. UGT1 is a glycosyltransferase enzyme...
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) / 08.01.2009

- Peter Krammer
  "Apoptosis in the immune system"
German Cancer Research Center, Heidelberg (DE) / 03.04.2008

- Timm Schneider
  "Signaling in hematopoiesis"
Institute of Stem Cell Research, Munich/Nuremberg (DE) / 15.04.2008

- Witold Filipowicz
  "Post-transcriptional gene regulation by microRNA"
Friedrich Miescher Institute for Biomedical Research, Basel (CH) / 08.05.2008

- Dimitris Kionis
  "Lymphoid organogenesis"
MRC National Institute for Medical Research, London (UK) / 20.05.2008

- Brigitte Stockinger
  "Inflammatory 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 Banerji
  "Harnessing dendritic cells for a better human health"
Baylor Institute for Immunology Research, INSERM 1499 – BIRC/INSERM/ANRS Center for Human Vaccines, Live Oak, Dallas (US) / 11.12.2008

- Federico Califori 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"
Institut Pasteur, Paris (FR) / 21.01.2009

- Klaus Rajewsky
  "B cell development"
Immune Disease Institute and Harvard Medical School, Boston, US / 10.02.2009
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) / 01.09.2007

- **Annette Oxenius**
  - "Immune control of Legionella pneumophila infection"
  - ETH Zurich, Institute of Microbiology, Zurich (CH) / 14.09.2007

- **Emmanuel Delamarche**
  - "H1N1N1 – 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 Rossi**
  - "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 Institute of Technology (EPFL), Lausanne (CH) / 1.02.2008

- **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

- **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 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, ZMBH, Münster, DE / 29.04.2009

- **Abel K. Abbas**
  - "Tolerance, autoimmunity and interleukin-2"
  - UCSF University of California, San Francisco (US) / 07.05.2009

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PHD COURSES & LECTURE SEMINARS

• Fabrizio Chiti
  “The determinants of protein aggregation: applications to animal models and to the bioinformatic analysis of the human proteome”
  University of Florence (IT) / 17.05.2008

• Olivier Michielin
  “Molecular modelling of protein: applications to cancer therapies”
  Swiss Institute of Bioinformatics and the Ludwig Institute for Cancer Research, University of Lausanne (CH) / 09.04.2008

• Gabriele Verani
  “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 naïve CD8+ T cells”
  International Center for Genetic Engineering and Biotechnology Area Science Park, Trieste (IT) / 01.06.2008

• Mario Roederer

• John Hartwig
  “Using different contractile systems to make and activate platelets”
  Brigham and Women’s Hospital, Harvard Medical School, Boston (US) / 18.06.2008

• Francesco Grussi
  “Regulation of bone remodeling by crosstalk between osteoclasts and T cells”
  University of Bern (CH) / 30.06.2008

• Luigi Calabuio
  “From Mad Cow disease to Creutzfeldt-Jacob disease: how NSM can help”
  Medical School of Pharmacy, The Universities of Kent and Greenwich at Medway (UK) / 04.07.2008

• Michel Gillier
  “Antimicrobial peptides in plasmacytid dendritic cells”
  Institut Pasteur, Paris (FR) / 01.08.2008

• Vincenzo Barnaba
  “The role of SIGIRR in primary human cells and rheumatoid arthritis: just an inhibitor of TLR signaling?”
  Kennedy Institute of Rheumatology, Imperial College London (UK) / 17.05.2008

• Mario Tschan
  “CD4+ cord blood cell-transplanted mice as a model for Epstein-Barr virus infection”
  University of Fribourg (CH) / 22.08.2008

• Hideo Ema
  “Regulation of bone remodeling by crosstalk between osteoclasts and T cells”
  University of Fribourg (CH) / 21.11.2008

• Fulvio Reggiori
  “Autophagosomes and other double-membrane vesicles: All the same?”
  Cell Microscopy Center and Institute of Biomembranes, University Medical Center (LMU), Munich (DE) / 27.02.2009

• Markus Aebi
  “Antigen presentation, priming and tissue-resident T cell memory to HSV-1”
  Dana-Farber Cancer Institute, Harvard Medical School, Boston (US) / 24.02.2009

• Stefan Drexler
  “Regulation of bone remodeling by crosstalk between osteoclasts and T cells”
  University of Konstanz, Kreuzlingen (CH) / 19.01.2009

• Joachim Hauber
  “The role of N-linked protein glycosylation: from yeast to humans – and back to prokaryotes”
  Institute of Medical Science, University of Konstanz (CH) / 20.01.2009

• Stefan Irwin
  “The role of miR-21 in lymphocyte biology”
  La Sapienza University of Rome (IT) / 01.06.2008

• Gabriele Verani
  “Cross-presentation, Immune-regulation & Antigen presentation, priming and tissue-resident T cell memory to HSV-1”
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• Vito Pistoia
  “Fragilakine: a novel chemok brunctor for a subset of human germinal center B cells”
  Giannina Gaslini Institute, Genova (IT) / 18.09.2008

• Vincento Bronte
  “Tumor-induced barriers to immune assault”
  Veneto Institute for Molecular Medicine, Padova (IT) / 30.10.2008

• Facundo D Batista
  “Early events in B cells response to antigen”
  Anderson Cancer Center, Houston (US) / 11.12.2008

• Marco Gattorno
  “Neutrophils as orchestrators of the immune response in Leishmania infections”
  Rosenberg Institute of Cancer Research, University of Melbourne (AU) / 12.06.2009

• Takayuki Ikeda
  “Modulation of dendritic cell functions by prostaglandin E2”
  University of Konstanz, Kreuzlingen (CH) / 20.01.2009

• William R Heath
  “Antigen presentation, priming and tissue-resident T cell memory to HSV-1”
  University of Melbourne (AU) / 12.06.2009
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ADMINISTRATION

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Vice Director
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Administrative Directors
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Guido Burati (Fidinam)

Administration
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Nadia Bernasconi
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Giossana Codoni
Davide Cotti
Lorenzo Dohà
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Michela Frascoli
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Dier Kingeien
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Martha Neagu
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Stefano Velpi
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Verena Calanca
Maria Gabriela Daniel
Blanca Fernandez
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Laura Perini
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Chiara Silacci
Tatiana Soldà
Fabrizia Varozetti

Facs Unit
David Jarnosay

Animal House Unit
Emanuele Cavadini
Andrea D'Ercole
Enrica Mira Catò

Support Staff
Ronnie Raccalà
Mauro Pasteris
Beatrice Pasteris

Note that some of the names listed above were present at the IRB for only part of the years 2007 - 2009.
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 11.5 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 million 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.
### Profit and Loss Account for the year 2008 (in CHF)

<table>
<thead>
<tr>
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<th>2008</th>
<th>2007</th>
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<tbody>
<tr>
<td>1. Personnel Costs</td>
<td>6'773'830</td>
<td>5'819'323</td>
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<tr>
<td>2. Consumables</td>
<td>2'135'474</td>
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<tr>
<td>3. Maintenance of Buildings and Equipment</td>
<td>548'218</td>
<td>531'760</td>
</tr>
<tr>
<td>4. Investments</td>
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<td>2'547'655</td>
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<tr>
<td>5. Amortizations</td>
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<tr>
<td>6. Rent and Related Costs</td>
<td>1'271'104</td>
<td>1'117'211</td>
</tr>
<tr>
<td>7. Administrative Costs and Various</td>
<td>94'7'45</td>
<td>95'7'43</td>
</tr>
<tr>
<td>8. Travel, Congresses and Guests</td>
<td>396'457</td>
<td>360'620</td>
</tr>
<tr>
<td>9. Financial charges</td>
<td>52'877</td>
<td>76'793</td>
</tr>
<tr>
<td>10. Various Costs for Research</td>
<td>672'311</td>
<td>754'790</td>
</tr>
<tr>
<td><strong>Total Costs</strong></td>
<td><strong>17'199'999</strong></td>
<td><strong>16'008'355</strong></td>
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### Balance Sheet as 31 of December 2008 (in CHF)

#### ASSETS

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>1. Liquidity</td>
<td>6'988'697</td>
<td>5'668'643</td>
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<tr>
<td>2. Various Receivables</td>
<td>1'731'867</td>
<td>1'385'480</td>
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<td>3. Temporary Receivables</td>
<td>69'4'109</td>
<td>1'200'135</td>
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<tr>
<td><strong>Current Assets</strong></td>
<td><strong>9'414'673</strong></td>
<td><strong>8'254'258</strong></td>
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<tr>
<td>4. Participations</td>
<td>12'000</td>
<td>12'000</td>
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<tr>
<td>5. Buildings</td>
<td>5'124'440</td>
<td>4'117'440</td>
</tr>
<tr>
<td>6. Furnishing &amp; Equipment</td>
<td>2'300'390</td>
<td>2'802'990</td>
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<tr>
<td><strong>Fixed Assets</strong></td>
<td><strong>7'836'940</strong></td>
<td><strong>8'917'340</strong></td>
</tr>
<tr>
<td><strong>Total Assets</strong></td>
<td><strong>17'161'613</strong></td>
<td><strong>17'186'198</strong></td>
</tr>
</tbody>
</table>

#### LIABILITIES

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>1. Debt for Delivery and Services</td>
<td>562'654</td>
<td>1'117'969</td>
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<tr>
<td>2. Accruals</td>
<td>523'553</td>
<td>341'051</td>
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<tr>
<td>3. Funds for Research Projects</td>
<td>1'869'065</td>
<td>1'816'102</td>
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<tr>
<td>4. Funds for Laboratories</td>
<td>1'655'908</td>
<td>1'359'909</td>
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<tr>
<td>5. Various Funds</td>
<td>1'228'480</td>
<td>950'000</td>
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<tr>
<td><strong>Current Liabilities</strong></td>
<td><strong>5'859'561</strong></td>
<td><strong>5'784'185</strong></td>
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<td>6. Long Term Loans</td>
<td>4'500'000</td>
<td>4'500'000</td>
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<tr>
<td><strong>Long Term Liabilities</strong></td>
<td><strong>4'500'000</strong></td>
<td><strong>4'500'000</strong></td>
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<tr>
<td>7. Capital Resources</td>
<td>6'902'213</td>
<td>6'846'794</td>
</tr>
<tr>
<td>8. Annual Result</td>
<td>10'039</td>
<td>95'220</td>
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<td><strong>Equity of the Foundation</strong></td>
<td><strong>6'912'053</strong></td>
<td><strong>6'902'013</strong></td>
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<tr>
<td><strong>Total Liabilities</strong></td>
<td><strong>17'161'613</strong></td>
<td><strong>17'186'198</strong></td>
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</tbody>
</table>


**ANNUAL RESULT** 10'039 95'220
Peer Reviewed Publications from July 2007


224. Clonal deletion of the human memory B-cell repertoire following infection and vaccination.
Pinnia D, Cotti D, Jamrozay D, Sallusto F, Lanzavecchia A.


226. Human B-cell memory.
Lanzavecchia A, Sallusto F.

227. FHLL, DCs, and NfTregs: team contra GYHDD?
Mare M G.
BLOOD. 2009;113:6267-6268.


230. Human naive and memory CD4+ T cell repertoires specific for naturally processed antigens analyzed using libraries of amplified T cells.
Geiger R, Dahan T, Lanzavecchia A, Sallusto F.

231. The concerted action of GM-CSF and Flt3-ligand on in vivo dendritic cell homeostasis.
Kangswon D, Schmid M A, Chui N, Okada-Ouhi A, Baumjohann D, Manz M G.


233. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells.
Dahan T, Geiger R, Jamrozay D, Lanzavecchia A, Sallusto F.

234. Potent inhibition of HIV-2 by TRIM5α-cyclophilin fusion proteins engineered from human components.

235. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells.
Dahan T, Geiger R, Jamrozay D, Lanzavecchia A, Sallusto F.

236. Human naive and memory CD4+ T cell repertoires specific for naturally processed antigens analyzed using libraries of amplified T cells.
Geiger R, Dahan T, Lanzavecchia A, Sallusto F.

237. The concerted action of GM-CSF and Flt3-ligand on in vivo dendritic cell homeostasis.
Kangswon D, Schmid M A, Chui N, Okada-Ouhi A, Baumjohann D, Manz M G.

Book Chapters


