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Institute for Research in Biomedicine

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Scientific Report
2011

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This Scientific Report covers the 2011 Research Activities
of the Institute for Research in Biomedicine (IRB)

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

Foreword by Giorgio Noseda
President of the Foundation Council

On March 29, 2012, the members of the Scientific Advisory Board of the Institute for Research in Biomedicine, Professors Anne O'Garra, London, Adriano Aguzzi, Zurich, Alberto Mantovani, Milan, Cesare Montecucco, Padua, met in Bellinzona to assess the results of the research activities achieved in 2011. Their Report to the Foundation Council states: "The Institute for Research in Biomedicine (IRB), Bellinzona, must be commended for its excellent success and research productivity in high impact areas. The success of their research is attested by impact publications in high profiles, cutting edge journals (312 publications since the founding of the IRB; 74 papers since January 2010: plus 3 book chapters since 2010) in the field of biomedical research. The average impact factor of all these publications is exceptional (11,2) without even having included the last two publications just newly accepted into Nature and Molecular Cell. In addition to these prolific, high quality publications, their recognition is also testified by editorials and commentaries including New England Journal of Medicine and Nature Reviews of Immunology, and Faculty of 1000. In addition, many of the researchers including F. Sallusto, A. Lanzavecchia and M. Molinari are constantly invited to speak at international conferences and schools, representing Bellinzona, which is now recognized as an international hub of immunology and technology research". Their words best witness the fact that the IRB continues to be among the leader centers in immunology research at the international level.

I'd like to refer in particular to the publication from David Corti, Antonio Lanzavecchia et al. "A Neutralizing Antibody Selected from Plasma Cells That Binds to Group 1 and Group 2 Influenza Hemagglutinins" that appeared in Science (August 18, 2011 - Volume 333, pp. 850-856). This article opens the perspective that the discovered monoclonal antibody can be used in the future for passive protection and might contribute to design a universal vaccine against Influenza as a result of its broad specificity and neutralizing power. The article has had the great honor of an editorial in The New England Journal of Medicine (Ch. J. Russell: "Stalking with Diversity in Universal Antibody" (October 20, 2011 - Volume 365, 16, pp. 1541-1542), which emphasized that "Although the task of obtaining a universal vaccine is daunting, the discovery of a universal antibody is a monumental first step".

The excellence of the IRB scientific research is regularly rewarded by allocation of several highly competitive grants (especially from the Swiss National Science Foundation and the European Union) to its researchers. In 2011, these competitive grants have totalized CHF 6.5 million. To these, were added two new project grants as well as one from the doctoral Programme (ProDoc) from the SNF, for a total of CHF 895'000 and 4 grants from the European Union (ADITEC, TIMER, IDAMS and ABIRISK) for a total of EURO 1'933'000. We are thus very grateful to our researchers, whom good applications to the national and international funding agencies contribute to 49% of the annual budget of the Institute.

Special thanks to the Foundations Horten, Jacob and Mäxi, which are supporting the IRB since years, and to the many other sponsors and private donors.

Lectures from invited speakers from other faculties in Switzerland, Europe and the United States within the frame of the "International PhD Programme in Immunology, Cell Biology and Biochemistry" are continuing, sometimes on a weekly or biweekly frequency. The programme allows to complete the post – graduate training of our researchers and PhD students. In 2011, thirty PhD students were performing their training at

the IRB and six have obtained their doctorate.
It should also be pointed out that the IRB, with about 80 employees, is a core center which appeals other research institutes hosted in its headquarters in Via Vela and Via Murate. In addition to the research laboratories of the IOSI (Oncology Institute of Italian Switzerland), which has now been renamed IOR (Institute of Oncology Research) and currently hosts about 30 employees, spaces were also allocated to the research laboratories of the Neurocenter of the Italian Switzerland, which currently employ 5 researchers. Thus, the "biological pole" of Bellinzona is composed of about 115 employees.
Negotiations are also underway to provide laboratory spaces in the ex-Gallera to PD Dr. Florian Bihl, gastroenterologist at the hospital S. Giovanni from Bellinzona, who will specifically study hepatitis B and C. This will in the future extend the field of immunology research to the infectious diseases of the liver.

In April, the city of Bellinzona has allocated an area of 13'000 m^2 of the former military camp for the construction of the new IRB headquarter, which is foreseen to host around 160-180 people. A referendum was launched against this decision last spring but the subsequent popular vote on June 19, 2011 has set out a unanimous decision in favour of the city: almost 9 in 10 people agreed with the transfer of the land (4,517 Yes against 564 No). In consequences, the changes of the Master plan was approved by the State Council and simultaneously rejected the appeal. Thus, a working committee has now been formed. It will organize an architectural competition, will choose the most suitable project, and will accompany the planning and construction of the new building, which should be ready in 4 to 5 years. This working committee is composed of Members of the Foundation Council, the Lawyer Paolo Agustoni and Dr. Carlo Maggini, and three representatives of the City of Bellinzona.

Another good news is the completed transfer of the Humabs company from the United States to Bellinzona, in Via Mirasole, company which is now called Humabs Biomed. A Research Agreement was signed with Humabs for the licensing and the subsequent commercial exploitation of patents for the production of monoclonal antibodies of human origin. This would permit the IRB to receive important royalties in the future. Humabs Biomed employs researchers in the IRBis headquarter in Via Murate and has granted the IRB with more than 6 million francs to continue its research. It is thus a strengthening of the biological pole of Bellinzona, a "win - win situation" and a virtuous example of "public - private partnership."

The cognitive capital fuels the creativity and innovation and it is a key factor for the economic and cultural development of a country. Helping to transform the Ticino in a more competitive and dynamic economy, means first of all investing in the production of knowledge. The IRB proactively contributes to this goal.

Prof. Dr. med. Giorgio Noseda

Bellinzona, May 2012

Foreword by Antonio Lanzavecchia

Director

At the beginning of the second decade of life, the Institute for Research in Biomedicine (IRB) has reached the main scientific objectives of becoming a research center for human immunology and biomedicine visible in the Swiss and international landscape.

The last three years have witnessed an increased integration of the IRB in the Swiss academic environment. The IRB is affiliated to the Università della Svizzera Italiana (USI) which is developing a Master Medical School in collaboration with the University of Zurich. The IRB has the potential to contribute significantly to the School in both teaching and training of students at the undergraduate and graduate level. The IRB group leaders are already involved in teaching activities as professors at the ETH Zurich, EPFL and at the universities of Bern and Zurich.

PhD students remain the driving force of the IRB and enjoy the open and friendly atmosphere of an English-speaking institute. At present, 26 graduate students work at IRB and 52 completed their training since 2000. Fresh doctorates as well as senior postdocs leaving the IRB have secured excellent positions in leading Institutions in Europe, America, Asia and Australia. The IRB PhD program course, supported by the Gustav & Ruth Jacob Foundation, offers a lecture series by world experts in the fields of cell biology, immunology and microbiology. In addition, block courses have been organized at IRB for ETH master students and for PhD students of Swiss universities in the framework of the ProDoc program. In 2011 the IRB organized a student retreat as well as the annual meeting of the Swiss Society for Allergology and Immunology in Lugano and a course of the European Academy of Dermatology and Venereology in Bellinzona.

The Institute currently hosts eight research groups led by Fabio Grassi, Antonio Lanzavecchia, Maurizio Molinari, Silvia Monticelli, Federica Sallusto, Marcus Thelen, Mariagrazia Uguccioni and Luca Varani. The recent research has produced a variety of interesting results. Among those are the role of ATP in balancing inflammatory and suppressor T cells; the isolation and characterization of broadly neutralizing antibodies including a pan-influenza A neutralizing antibody; the identification of a novel compartment of the endoplasmic reticulum which is used for viral replication, and the identification of a novel role for IL-1 in determining the inflammatory properties of Th17 cells. Other studies revealed novel aspects of leukocyte migration such as the crosstalk between HMGB-1 and chemokines, and the role of the decoy receptor CXCR7. A detailed description of the ongoing research in the eight laboratories of the IRB is found in this report.

In the last year the IRB has strongly improved its core facilities that not only support the internal activities but are also accessible to other researchers in the Ticino area. These include the cell sorting and imaging facility, which has been updated and is now supervised by two dedicated researchers, and the new protein production facility which allows IRB scientists to take full advantage of their discoveries in various fields ranging from antigen identification and epitope mapping to protein-protein interaction. These new developments have been possible through the generous support from the Mäxi Foundation which is also supporting the establishment of a new group led by Santiago Fernández González who will work in the field of antigen and cell trafficking in infectious diseases, and will establish a cutting edge two photon microscopy laboratory. IRB scientists have established an effective network of collaborations with leading institutions worldwide. The grants received from the Swiss National Science Foundation, the European Union, the European Re-

search Council, the Bill & Melinda Gates Foundation, and the US National Institutes of Health, attest to their success. Research programs have also been supported by the Swiss Vaccine Research Institute (SVRI) and by the Institute for Arthritis Research (IAR). The IRB has also been an effective incubator for Biotech companies. The technologies to make human monoclonal antibodies were licensed by the IRB in 2004 to a startup company, Humabs, that has moved in December 2011 from the USA to Switzerland and has established office and laboratories in Bellinzona. A collaboration agreement between IRB and Humabs has provided over the years a continuous support to the research and may result in a royalty stream in years to come.

A special thanks goes to the members of the IRB Foundation Council, in particular to the President Giorgio Noseda, for the vision of developing an institute dedicated to basic research in biomedicine and for their continuous support. 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, May 2012

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RESEARCH PROJECTS

SECTION 1

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Fabio Grassi

T Cell Development

Fabio Grassi

Fabio Grassi earned his degree in Medicine at the University of Pavia in 1985 and a Ph.D. in Microbiology at the University of Milan in 1993. He was a Anna Villa Rusconi fellow at the University of Umeä in Sweden (1988), post-doctoral fellow at the Institut Pasteur in Paris (1989-1993), assistant professor at San Raffaele Scientific Institute in Milan (1994-1998), Marie Curie fellow at Hôpital Necker in Paris (1998-2000) and Special Fellow of the Leukemia & Lymphoma Society at Dana Farber Cancer Institute, Harvard Medical School in Boston (2000-2002). He is associate professor of Biology at the Medical School of the University of Milan. In September 2002, he joined the IRB as head of the T Cell Development lab. His research is focused on various aspects of T cell physiology, including protein and membrane trafficking, signal transduction, control of cell growth and intercellular communication during T cell development and in immunopathological conditions.

Research Focus

Adenosine-triphosphate (ATP) is the source of chemical energy for the majority of cellular functions, serves as a substrate in signal transduction pathways and is incorporated into nucleic acids during DNA replication and transcription. ATP can also be released by eukaryotic cells and act as a signalling molecule in an autocrine/paracrine fashion by activating purinergic P2 receptors in the plasma membrane. The research in the lab focuses on the purinergic regulation of T cell physiology, namely T cell receptor (TCR) driven signalling, gene expression and fate determination at various stages of development. Cellular metabolism regulates T cell function and differentiation, and its targeting might be exploited to manipulate adaptive immune responses. T cell stimulation by cognate antigen determines early Ca^{2+} influx, which cause a burst of oxidative ATP synthesis in the mitochondria. ATP released upon TCR triggering contributes to the activation of mitogen-activated protein kinase (MAPK) through P2X receptors in an autocrine manner. Inhibition of this autocrine purinergic signalling determines T cell anergy and favors polarization of naïve $CD4^+$ cells toward the immunosuppressive regulatory T cell fate. Therefore, pharmacological P2X antagonism might alter adaptive immune system responsiveness. The experiments conducted in the lab are aimed at understanding the role of purinergic signalling in conditioning T cell function in distinct tissue microenvironments.

Team

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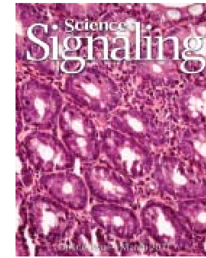
Members: Vanessa Cornacchione, Diploma student - Caterina Elisa Faliti, PhD student - Michela Frascoli, PhD student - Jessica Marcandalli, Diploma student - Lisa Perruzza, PhD student - Michele Proietti, MD, PhD - Tanja Rezzonico Jost, Technician - Rosita Rigoni, Diploma student - Andrea Romagnani, PhD student.

Purinergic regulation of immunosuppressive regulatory T cells

Ursula Schenk, Michela Frascoli, Michele Proietti, Caterina Faliti, Vanessa Cornacchione and Fabio Grassi

Extracellular nucleotides are pleiotropic regulators of mammalian cell function. Adenosine triphosphate (ATP) can be released by eukaryotic cells and activate purinergic receptors in the plasma membrane, known as P2. Two classes of P2 receptors exist in eukaryotic cells. The first consists of P2Y receptors, which are metabotropic, i.e. coupled to heterotrimeric guanine nucleotide-binding protein (G-protein) and modulate mainly intracellular calcium as well as cyclic AMP levels; the second is composed of P2X receptors, which are ionotropic, i.e. ligand-gated cation-permeable channels that open when bound to ATP. ATP released from $CD4^+$ helper T cells upon stimulation of the T cell receptor (TCR) contributes, in an autocrine manner, to the activation of mitogen-activated protein kinase (MAPK) signalling through purinergic P2X receptors. Increased expression of *p2rx7*, which encodes the purinergic receptor P2X7, is part of the transcriptional signature of immunosuppressive $CD4^+CD25^+$ regulatory T cells (T_{regs}). We have shown that the activation of P2X7 by ATP inhibits the suppressive potential and stability of T_{regs}. The inflammatory cytokine interleukin-6 (IL-6) increased ATP synthesis and P2X7-mediated signalling in T_{regs}, which induced their conversion to IL-17–secreting T helper 17 (T_H17) effector cells *in vivo* (Figure 1). Moreover, pharmacological antagonism of P2X receptors promoted the cell-autonomous conversion of naïve $CD4^+$ T cells into T_{regs} after TCR stimulation. Thus, ATP acts as an autocrine factor that integrates stimuli from the microenvironment and cellular energetics to tune the developmental and immunosuppressive program of the T cell during adaptive immune responses.



* *Schenk U. et al.* *Sci Signal. 2011, 4:ra12.*

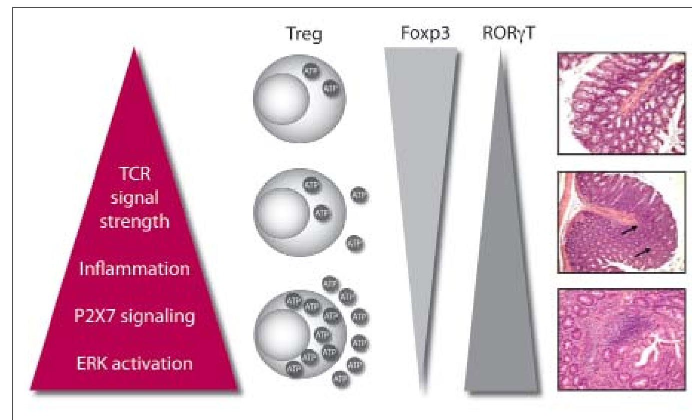


Figure 1. Modulation of regulatory T cells immunosuppressive function by ATP. *Increasing TCR signal strength, inflammatory mediators, P2X7 signalling or ERK activation correlate with progressive increases in ATP levels in T_{regs}, which result in diminished and increased abundance of Foxp3 and Rorc transcripts, respectively. Foxp3 and RORγt define the relevant proteins. This results in progressive increases of IL-17-secreting $CD4^+$ T cells (not shown) and inflammatory tissue damage, as displayed in colon sections from immuno-deficient mice injected with syngenic naïve $CD4^+$ T cells together with $p2x7^{-/-}$ T_{regs} (normal colon, top panel) or wild-type T_{regs} (moderately inflamed colon, middle panel) or alone (severely inflamed colon, lower panel). Arrows in the middle panel indicate inflammatory cells.*

Regulation of hematopoietic stem cell cycling activity by ATP

Anna Casati, Michela Frascoli, Michele Proietti and Fabio Grassi

Hematopoietic stem cells (HSCs) constitute a minute quiescent and self-renewing cell population with the potential to enter cell cycle and differentiate into progenitors of different cell lineages. Exogenous stimuli can induce HSC proliferation and differentiation into lineage-committed progenitors. For example, stimulation of toll-like receptors (TLRs) expressed in hematopoietic progenitors was shown to trigger cell cycle entry as well as myeloid differentiation. Extracellular nucleotides were shown to stimulate the proliferation of human HSCs. We asked whether an autocrine purinergic loop might regulate the cell cycling activity of murine HSCs after stimulation with cytokines or ligands of innate immune system receptors expressed in HSCs. We have shown that in HSCs, ATP is stored in vesicles and released in a calcium-sensitive manner (Figure 2). HSCs express ATP-responsive P2X receptors and *in vitro* pharmacological P2X antagonism restrained hematopoietic progenitors proliferation, but not myeloid differentiation. In mice suffering from chronic inflammation, HSCs were significantly expanded and their cycling activity was sensitive to treatment with the P2X antagonist periodate-oxidized 2,3-dialdehyde ATP. Our results indicate that ATP acts as an autocrine stimulus, which positively influences HSC proliferation and regulates the population size of uncommitted hematopoietic progenitors.

Casati A. et al. * *Cell Death Differ. 2011, 18:396-404.*

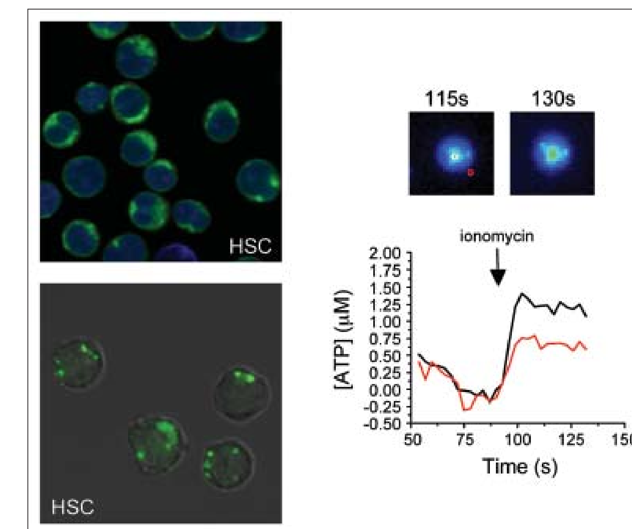


Figure 2. Vesicular storage and calcium-sensitive release of ATP in hematopoietic stem cells. *Left panels: Sorted lineage-negative, c-kit$^+$, Sca-1$^+$ CD34$^-$ hematopoietic stem cells (HSCs) were stained with the nucleotide-binding compound quinacrine and nuclear red (DRAQ5), and analyzed in live imaging confocal microscopy. Quinacrine-positive staining is detected in the cytoplasm (top panel). Punctate pattern of quinacrine-stained cells in phase-contrast image (lower panel). Right panels: ATP release from HSC treated with the calcium ionophore ionomycin (1 μM) measured as an increase in NADPH fluorescence generated by a two-enzyme assay. Pseudocolor images at different times (top panels) and ATP levels in a region of interest (ROI) placed above the cell (black line) or in proximity of the same cell (red line) show the increased fluorescence in the HSC pericellular region. The increase in ATP in ROI above the cells (black line) is indicative of calcium-induced mitochondrial NADPH synthesis (lower panel).*

Role of purinergic P2X7 receptor in T cell lineage choice

Michela Frascoli, Jessica Marcandalli, Andrea Romagnani and Fabio Grassi

In immature T cells, lineage choice during development in the thymus is dictated by T cell receptor (TCR) signal strength with increasing strength resulting in induction of the γδ differentiation program. In fact, artificial reduction of γδTCR signalling was shown to divert γδTCR expressing cells toward the γδ fate. Expression of the purinergic receptor P2X7 was selectively increased in immature γδ $CD25^+$ cells. Analogous to mature T cells, these cells released ATP following TCR stimulation. Treatment of E14 fetal thymus organ cultures (FTOC) with a P2X7 receptor antagonist, periodate-oxidized 2,3-dialdehyde ATP, resulted in the generation of aberrant $CD4^+8^+$ cells (e.g. αβ committed) expressing γδTCR. This phenomenon correlated with impaired ERK phosphorylation in *p2rx7* knock-out immature γδ thymocytes upon γδTCR stimulation. Our results indicate that ATP mediated P2X7 signalling influences T cell lineage choice during thymic development by regulating TCR signal strength.

Induced thymus development as a therapeutic approach in Omenn Syndrome

Lisa Perruzza and Fabio Grassi

Omenn syndrome (OS) is a combined immunodeficiency associated with generalized erythrodermia, alopecia, lymphadenopathy, hepatosplenomegaly, and chronic diarrhea. In most cases, OS results from hypomorphic mutations of the *rag1* and *rag2* genes that decrease, but do not completely abolish, V(D)J recombination activity. A common feature associated with these mutations is the presence of an oligoclonal T cell repertoire, supporting the idea that OS is caused at least in part by a lymphopenic condition, which leads to dysregulated homeostatic proliferation of $CD4^+$ T cells. T lymphocytes in patients with OS display an activated/memory phenotype, have an extremely limited TCR repertoire and infiltrate target organs, resulting in tissue damage. OS patients are threatened both by severe infections (due to immune deficiency) and organ damage (due to "autoimmune" T cell-mediated reactions). Because of this complex phenotype, treatment of OS requires immune suppression and hematopoietic stem cell transplantation. The $rag2^{R229Q/R229Q}$ mouse carries the *rag2* R229Q mutation identified in patients with OS and is thus a model for the human disease. The thymus of these mice is very small, lacks a cortico-medullary demarcation and is devoid of Hassall's-like clusters. There is a significant block at the double negative DN3 ($CD44^-$ $CD25^+$) stage of T cell development. Although the number of double positive (DP) cells is extremely reduced, a few single positive (SP) thymocytes are consistently detected. Of note, the mTEC population mainly responsible for the negative selection process is poorly represented, resulting in a markedly reduced expression of Aire, a key player in governing central tolerance. The subverted thymic architecture that causes abnormal crosstalk between thymocytes and epithelial cells, could be responsible for the generation of autoreactive T cells that infiltrate peripheral organs. In $rag2^{R229Q/R229Q}$ mice, the impaired generation of the pre-TCR, which is crucial for proper development of thymic epithelium, determines the block of thymocyte development at the DN3 stage. Anti-CD3 administration in $rag2^{-/-}$ mice induces DN thymocytes to proliferate into DP cells and promotes a succession of events normally driven by pre-TCR signalling. Based on these findings and to gain insight into the potential role of thymus atrophy in the pathogenesis of OS, we investigated whether exposure of $rag2^{R229Q/R229Q}$ T cell progenitors to a "normal" thymic microenvironment during TCR repertoire selection might affect the $rag2^{R229Q/R229Q}$ mice phenotype. Chimeric $rag2^{-/-}$ mice, in which hypomorphic ($rag2^{R229Q/R229Q}$) progenitors developed in $rag2^{-/-}$ thymus induced by anti-CD3 treatment, displayed significant amelioration of T cell-mediated immunopathology with respect to non-injected chimeric mice. Therefore, we are assessing the potential efficacy of early "thymopoietic" anti-CD3 treatment in experimental OS.

Pharmacological purinergic antagonism in T-cell acute lymphoblastic leukaemia (T-ALL)

Tanja Rezzonico-Jost, Lisa Perruzza and Fabio Grassi

T-cell acute lymphoblastic leukaemia (T-ALL) is characterized by a high risk of central nervous system (CNS) infiltration by leukemic cells. T-ALL patients with an increased risk of CNS relapse, in addition to intensified intrathecal chemotherapy, receive prophylactic cranial irradiation, which can cause severe complications. Therefore, therapies that improve efficacy and reduce side-effects remain a long-term objective in the treatment of CNS relapse in T-ALL. Since P2 receptors are important in regulating chemokine synthesis in the CNS and may be important in mediating communication with hematopoietic cells, we are currently investigating whether pharmacological purinergic antagonism might represent a therapeutic option in T-ALL.

Development of an implantable system to monitor inflammation and metabolism

Tanja Rezzonico Jost, Michele Proietti, Rosita Rigoni, Andrea Romagnani and Fabio Grassi

The aim of this project is to develop an implantable biochip system to investigate the complexity of drugs/biomarkers relationships in chronic inflammatory diseases. To reach this goal, a multidisciplinary approach is needed because the system requires: (i) The development of an innovative sensor to detect an array of drugs, including nanotechnology and system level integration to improve sensor specificity; (ii) The development of new micro-electronics technology to decrease chip size for implantation in mice as well as a convenient chip remote powering data transmission; (iii) Testing in murine models of chronic inflammation; (iv) Investigating the pharmacokinetics of biochemical enzymes-substrates to identify the best cytochrome P450 isoforms, out of more than 3,000 possibilities, to be integrated onto the biochip in order to ensure the detection of those exogenous and endogenous compounds which are relevant for the specified medical application. To best address all of these multidisciplinary demands, the project partnership includes experts in: (i) Nano-sensing, with a special focus on P450 biosensors (S.Carrara/EPFL), (ii) Chip fabrication with focus on implantable systems (Dehollain /EPFL), (iii) Biomarker variations (our group), (iv) Pharmacokinetics (Von Mandach/University of Zurich Hospital)

Funding

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Purinergic signalling in T cell physiology
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Swiss National Science Foundation
Innovative enabling micro-nano-biotechnologies for implantable systems in molecular medicine and personalized therapy
CRSII2-127547 / 2010-2013

Nano-tera.ch
Implantable/wearable system for on-line monitoring of human metabolic conditions
(Acronym: i-IRONIC)
841-402 / 2010-2013

Swiss Cancer League
Purinergic signalling in the pathophysiology of central nervous system infiltration in T-cell leukaemia
KFS 02445-08-2009 / 2010-2012

Fondazione Ticinese per la Ricerca sul Cancro
Purinergic signalling in T-cell acute lymphoblastic leukaemia
2010-2011

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Publications

Cell-autonomous regulation of hematopoietic stem cell cycling activity by ATP.
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Nat Immunol. 2011; 12: 870-8.

Lectures and Seminars

University of Perugia
Department of Experimental Medicine and Biochemical Sciences
Seminar “T cell regulation by extracellular ATP”
Perugia (IT) / 11.02.2011

University of Turin
Department of Genetics, Biology and Biochemistry
Lecture Series in Experimental Medicine
Seminar “T cell functional regulation by purinergic stimulation”
Turin (IT) / 23.02.2011

Keystone Symposia,
Immunoregulatory Networks Conference
“Inhibition of regulatory T cell function and generation by purinergic P2X receptors”
Breckenridge, CO (USA) / 03.04.2011

6th Leukocyte Signal Transduction Workshop
“Purinergic modulation of regulatory T cell lineage stability”
Chania, Crete (GR) / 08.06.2011

Ludwig-Maximilians University Munich
Walther-Straub-Institute for Pharmacology and Toxicology
Seminar “Purinergic modulation of regulatory T cells”
Munich (DE) / 21.09.2011

Joint International Congress of Cell Transplant Society & Xenotransplantation Society
“Purinergic modulation of tissue specific regulatory T cell”
Miami, FL (US) / 26.10.2011

Antonio Lanzavecchia
Immune Regulation

Antonio Lanzavecchia

Antonio Lanzavecchia earned a degree in Medicine at the University of Pavia where he specialized in Paediatrics and in Infectious Diseases. From 1983 to 1999, he was a Member of the Basel Institute for Immunology and since 1999 he is the founding Director of the Institute for Research in Biomedicine in Bellinzona. He has been teaching at the University of Genoa and Siena and since 2009 is Professor of Human Immunology at the Swiss Federal Institute of Technology Zurich. He is Member of the European Molecular Biology Organization (EMBO) and Fellow of the Royal College of Physicians. Awarded the EMBO medal in 1988 and the Cloëtta prize in 1999, Antonio Lanzavecchia has published more than 250 papers. His research has covered several aspects of human immunology: from antigen processing and presentation to dendritic cell biology and from lymphocyte activation and trafficking to T and B cell memory.

Research Focus

In recent years our research has focused on the biology of human memory B cells and plasma cells and on the characterization of the human antibody response to infectious agents. In particular we study the dynamics of memory B cells and plasma cells in the steady state and in the secondary response with the aim of defining the mechanisms that determine plasma cell longevity and maintain serum antibody levels. We have developed and continuously refine high throughput cell culture based methods to isolate human monoclonal antibodies from memory B cells and plasma cells. These methods are used to study the dynamics and selection of B cells specific for pathogens or self antigens and to isolate potent neutralizing antibodies. Through the analysis of the specificity of such antibodies we can identify unique conserved epitopes that can be used for vaccine design, an approach that we termed "analytic vaccinology".

Team

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A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins

Davide Corti, Giosiana Codoni, Annalisa Macagno, David Jarrossay, Debora Pinna, Andrea Minola, Chiara Silacci, Blanca M. Fernandez-Rodriguez, Isabella Giacchetto-Sasselli, Federica Sallusto and Antonio Lanzavecchia

The isolation of broadly neutralizing antibodies against influenza A viruses has been a long-sought goal for therapeutic approaches and vaccine design. Using a single-cell culture method for screening large numbers of human plasma cells, we isolated from the same donor in three separate occasions a neutralizing monoclonal antibody that recognized the hemagglutinin (HA) glycoprotein of all 16 subtypes and neutralized both group 1 and group 2 influenza A viruses. Passive transfer of this antibody conferred protection to mice and ferrets against H1N1, H3N2 and H5N1 influenza viruses. An engineered FI6 antibody lacking complement and FcR binding showed reduce efficacy suggesting that *in vivo* protection is also mediated by lysis of infected cells. The antibody reverted to the germline sequence only to group 1 HAs, indicating that the reactivity with Group 2 HAs was acquired as a function of somatic mutations. Complexes with HAs from the group 1 H1 and the group 2 H3 subtypes analyzed by x-ray crystallography showed that the antibody bound to a conserved quaternary epitope in the F subdomain. FI6 binding is mediated by both VH and VL CDRs, with a prominent contribution of the long HCDR3 and of the heavily mutated LCDR1, which accommodate different Group-specific conformations of the Trp-21 loop as well as the conserved Asn-38 glycan present in Group 2 HAs (Figure 1). This is the first example of an HA-specific pan influenza A neutralizing antibody that may be used for passive protection and to inform vaccine design because of its broad specificity and neutralization potency.

* *Corti et al*
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This work was done in collaboration with John Skehel and Steve Gamblin, MRC, National Institute for Medical Research, Mill Hill, London, UK; Nigel Temperton, University of Kent, UK; Johannes Langedijk, Pepscan Therapeutics Lelystad, NL; and Fabrizia Vanzetta and Gloria Agatic, Humabs BioMed, Bellinzona, CH.

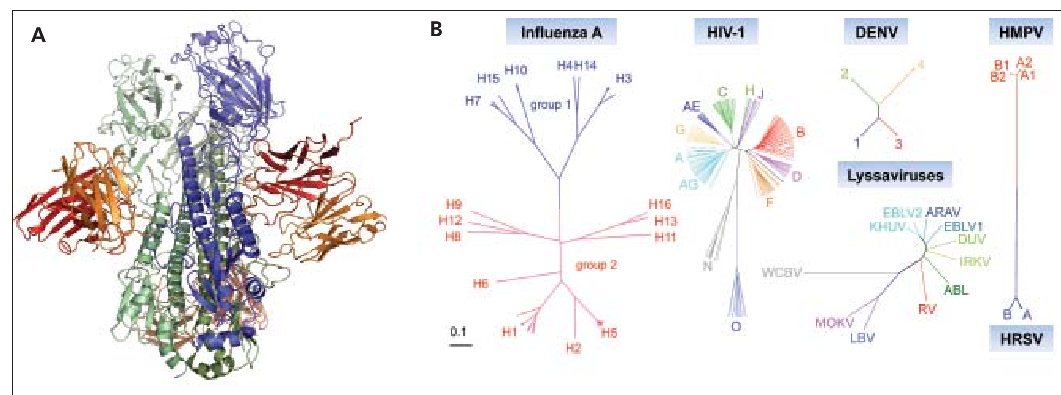

Figure 1:
A. Crystal structure of FI6 Fab fragments bound to influenza hemagglutinin from the H1 swine pandemic virus. B. Diversity of glycoproteins of human RNA viruses.

Heterosubtypic antibody responses elicited by seasonal and pandemic influenza vaccines

Davide Corti, Chiara Silacci, Debora Pinna and Antonio Lanzavecchia

We have previously shown that heterosubtypic neutralizing antibodies are produced by some individuals in response to seasonal influenza vaccines. This antibody response is directed primarily against conserved epitopes present in the stem region of the influenza HA of group 1 viruses. To analyze the heterosubtypic antibody response elicited by monovalent pandemic (n=114) or trivalent seasonal (n=59) influenza vaccination we measured the capacity of serum to inhibit the binding of know heterosubtypic monoclonal antibodies to the stem region of H5 and H7 HAs, representative of Group 1 and Group 2 HAs respectively. Most donors (85%) had a detectable titer of Group 1 heterosubtypic antibodies before vaccination, which increased significantly 28 days after vaccination (80% binding displacement titers, BD80, before vaccination 1/145 vs. 1/417 after vaccination, $p<0.0001$). BD80 values correlated with the microneutralization (MN) titers of a lentiviral pseudotype expressing the same H5 but not with the titers of hemagglutination inhibition titers to the vaccine strain. In contrast, only 27% of the donors had detectable levels of Group 2 heterosubtypic serum antibodies before vaccination and overall showed lower BD80 titers (BD80 1/47 against group 2 versus 1/170 against group 1, $p<0.0001$). No significant increase in heterosubtypic antibodies to group 2 was observed following pandemic and even seasonal vaccination in spite of the fact that seasonal vaccine contains a Group 2 virus. Our results show that the pandemic H1N1 vaccine as well as the trivalent seasonal vaccine can boost preexisting heterosubtypic memory B cells specific for Group 1 HAs, but only rarely Group 2 or pan-influenza specific responses, but the pandemic H1N1 vaccine was more efficient than the seasonal strain in inducing both strain-specific and hetero-subtypic antibodies.

This work was done in collaboration with Elisa Vicenzi, HSR, Milan, IT.

Monoclonal antibodies with neutralizing breadth from HIV-1-infected individuals

Davide Corti, Blanca M. Fernandez-Rodriguez, Chiara Silacci, Debora Pinna, David Jarrossay and Antonio Lanzavecchia

The isolation and characterization of human monoclonal antibodies that neutralize a broad spectrum of primary HIV-1 isolates is central to the design of effective antibody-based vaccine strategies. From memory B cells of HIV infected individuals we isolated several monoclonal antibodies that were mapped to different sites of the Env glycoprotein. One mAb (HJ16) binds to a "core epitope" of the CD4 binding site on gp120 and neutralized a multi-clade panel of Tier-2 HIV-1 pseudoviruses. This antibody is highly mutated and the germline reverted antibody does not bindo to gp120 suggesting a role for crossreactive stimulation and affinity maturation. A second mAb (HGN194) bound a conserved epitope in the V3 crown and neutralized all Tier-1 and a proportion of Tier-2 pseudoviruses tested, irrespective of clade. This antibody conferred sterilizing immunity to macaques challenged mucosally with a clade C SHIV isolate while inducing gag-specific CD4+ and CD8+ T cells. Further *in vivo* experiments are ongoing to assess the role of antibody effector function in protection from mucosal challenge. A third mAb (HK20) showed a very broad spectrum of neutralizing activity, particularly as a Fab or scFv fragment. This antibody showed assay- and size- dependent selectivity in its activity recognized a highly conserved epitope in the HR-1 region of gp41 as shown by the crystal structure of the HK20 Fab in complex with a gp41 mimetic 5-Helix (Figure 2). Given the

recent reports on broadly neutralizing antibodies that target conformational epitopes that are not present on recombinant proteins, we developed a new screening strategy based on a micro neutralization assay and further screenings are ongoing.

* *Ruprecht C. et al.* *PLoS ONE 2011, 6:e18207.*

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* *Balla-Jhagjhoorsingh S. et al.* *PLoS ONE 2011, 6:e25488.*

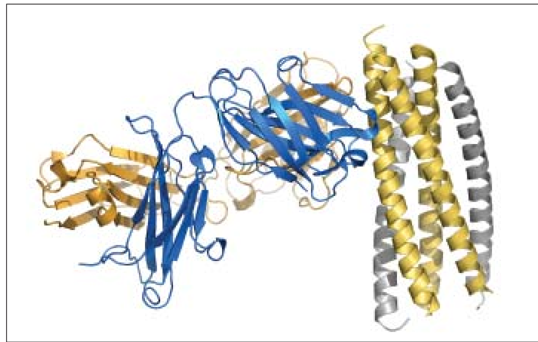

Figure 2.
Crystal structure of HK20 Fab fragments bound to the heptad repeat 1 of HIV gp41.

Fc-dependent phagocytosis and killing of P. falciparum by human monoclonal antibodie

Janine Stubbs and Antonio Lanzavecchia

It is widely accepted that antibodies against the human parasitic pathogen Plasmodium falciparum protect the host from severe malaria and death. However, there is a continuing need for the development of *in vitro* correlate assays of immune protection. To this end, the capacity of human monoclonal antibodies in eliciting phagocytosis and parasite growth inhibition via Fcγ receptor-dependent mechanisms was explored. In examining the extent to which sequence diversity in merozoite surface protein 2 (MSP2) results in the evasion of antibody responses, an unexpectedly high level of heterologous function was measured for allele-specific human antibodies. The dependence on Fcγ receptors for opsonic phagocytosis and monocyte-mediated antibody-dependent parasite inhibition was demonstrated by the mutation of the Fc domain of monoclonal antibodies against both MSP2 and a novel vaccine candidate, peptide 27 from the gene PFF0165c. These flow cytometry-based functional assays are expected to be useful for assessing immunity in naturally infected and vaccinated individuals and for prioritizing among blood-stage antigens for inclusion in blood-stage vaccines. This work and others from our lab has provided proof of principle that the isolation of human monoclonal antibodies can advance our knowledge of the immune response to the malaria parasite and help identify critical molecular targets. Unfortunately, due to lack of blood supply, we are unable to continue this promising line of research.

* *Stubbs J. et al.* *Infect Immun. 2011, 79:1143-52.*

This work was done in collaboration with Giampietro Corradin, University of Lausanne, CH.

Identificiation of cellular factors involved in T cell proliferation and differentiation through shRNA and metabolic screens

Roger Geiger and Antonio Lanzavecchia

The aim of the project is to use two complementary approaches in physiologically relevant cellular systems to achieve a thorough basic molecular understanding of the human T cell biology and to define principles of regulation of T cell proliferation and differentiation. In the first, genomic approach, we will use an unbiased, genome-wide screening method based on the shRNA technology, to systematically analyze the involvement of cellular factors in the initial events of naïve T cell priming and regulation of cell proliferation. From this functional screening – performed in primary naïve T cells isolated from peripheral blood of healthy adult donors – we will identify genes that positively or negatively regulate cell proliferation and differentiation. In the second, metabolomic approach, we will use recently developed analytical and computational tools to analyze the metabolic profile of different types of effector and memory T cells in different activation states. The metabolic profile of a cell is determined by the expression and the properties of various enzymes and provides integrative information on metabolic pathways that are associated with proliferation and differentiation. Based on the outcomes, we will test the causality and relevance of the identified metabolic pathways directly in T cells by RNA interference or through the use of inhibitors.

This work is done in collaboration with Federica Sallusto, IRB and Nicola Zamboni, ETH Zurich, CH.

Protecting and pathogenic responses to Dengue virus

Martina Beltramello and Antonio Lanzavecchia

Antibodies protect against homologous Dengue virus (DENV) infection but can precipitate severe dengue by promoting heterotypic virus entry via Fcγ receptors (FcγR). We previously reported that human monoclonal antibodies to envelope (E) protein domain III (DIII) potently neutralized DENV infection and were either serotype specific or cross-reactive with two or three but never with all four DENV serotypes. In contrast, DI/DII- and prM-reactive mAbs neutralized poorly but most completely cross-reacted with the four DENV serotypes. Furthermore we could confirm that all antibodies enhanced infection at sub-neutralizing concentrations. Three mAbs targeting distinct epitopes on the four DENV serotypes and engineered to prevent FcgammaR binding did not enhance infection and neutralized DENV *in vitro* and *in vivo* as post-exposure therapy in a mouse model of lethal DENV infection. These findings illustrate the potential for an antibody-based therapy to control severe dengue and reveal an unexpected degree of cross-reactivity in human antibodies against DI/II. Based on our results we hypothesize that the potently neutralizing DIII-specific antibodies protect form homologous but not from heterologous challenge, while the poorly neutralizing DI/II- and PrM-specific antibodies are the primary culprit for enhancing heterologous infection. We are currently testing this hypothesis by analyzing the antibodies produced by mice immunized with recombinant proteins.

De Alwis R. et al. * *PLoS Negl Trop Dis 2011, 5 :e1188*

This work was done in collaboration with Federica Sallusto and Luca Varani, IRB, Bellinzona; Cameron Simmons, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam; Felix Rey, Institut Pasteur, Paris, FR; Michael S Diamond, Washington University School of Medicine, St. Louis, MO, US; Eva Harris, University of California, Berkeley, CA, US; and Aravinda de Silva, University of North Carolina, Chapel Hill, NC, US.

Clonal dynamics of human memory B cells and plasma cells upon repeated immunizations

Leontios Pappas, Blanca Fernandez Rodriguez, Chiara Silacci, Davide Corti and Antonio Lanzavecchia

By combining high throughput cell culture based methods with molecular analysis of isolated antibodies we set out to study the clonal dynamics of a T-dependent antibody responses and the intraclonal selection of antibody variants. In particular we aim to define the precursor-product relationship between preexisting memory B cells and the plasma cells and memory B cells generated following a booster immunization. To reduce the complexity of the antibody response analyzed we focus the analysis on heterosubtypic antibodies that recognize conserved epitopes in the stem region of group 1 influenza HAs. These antibodies use primarily, although not exclusively, VH1-69 but somatic mutations are important to increase affinity. We have extensively cloned memory B cells and plasma cells from a donor from which serial samples were collected before and after vaccination over 4 consecutive years. The sequencing of VH and VL genes from plasma cells and memory B cells allows the precise delineation of family trees and the analysis of the specificity and affinity of the corresponding antibodies. We have currently sequenced >200 cells that had been grouped into 15 clones which show distinct dynamics following repetitive antigenic stimulation.

Can serotherapy treat rabies virus infection?

Andrea Minola, Blanca Fernandez Gonzales, Isabella Giacchetto-Sasselli, Davide Corti and Antonio Lanzavecchia

Rabies is a life threatening infectious disease caused by lyssaviruses belonging to 7 distinct genotypes that is transmitted by the bite of infected mammals and causes ~60.000 deaths per year. The only available treatment is post exposure prophylaxis (PEP) that consists in the administration of low amounts of rabies hyper immune globulins at the site of the bite together with an inactivated rabies genotype 1 vaccine. PEP is effective if administered promptly within 24 hours after the bite but is not effective when the virus has already reached the CNS. In addition PEP is less effective for non-genotype 1 lyssaviruses. From donors vaccinated with the genotype 1 vaccine we isolated hundreds of monoclonal antibodies and among these we selected two that potently and broadly neutralize viruses representative all 7 lyssavirus genotypes including newly isolated eurasian bat viruses. The two antibodies bind to distinct antigenic sites and are therefore suitable candidates for the a cocktail that can neutralize most lyssavirus infections including those on which PEP is not effective. *In vivo* experiments are ongoing in Syrian Hamsters to assess the therapeutic potential of these human monoclonal antibody cocktail.
This work was done in collaboration with Paola De Benedictis and Ilaria Capua, IZV, Legnaro, IT; Herve Bourhy, Institut Pasteur, Paris, FR; Ed Wright and Robin Weiss, UCL London, UK; Fabrizia Vanzetta and Gloria Agatic, Humabs BioMed SA, Bellinzona, CH.

Characterization of monoclonal antibodies to GM-CSF from patients with pulmonary alveolar proteinosis

Luca Piccoli, Blanca Fernandez Rodriguez, Davide Corti and Antonio Lanzavecchia

Pulmonary alveolar proteinosis (PAP) is a rare severe autoimmune disease caused by autoantibodies that neutralize GM-CSF causing the loss of alveolar macrophages and the accumulation of lipoproteinaceous material within alveoli. By immortalizing memory B cells from 4 PAP patients we isolated >40 IgG monoclonal antibodies that bind to human GM-CSF with high affinity. The VH and VL genes are highly mutated suggesting that this antibody response is T-dependent and we are currently producing the germlined version to investigate the mechanism of antigenic selection. Surprisingly, none of these antibodies showed strong GM-SF neutralizing activity, in spite of high neutralizing activity of the patient's serum. We are currently expanding the panel of antibodies and constructing an antigenic map of GM-CSF using cross-competition studies performed by surface plasmon resonance. Further studies aim at characterizing the T cell response to GM-CSF.

This work was done in collaboration with Federica Sallusto, Luca Varani and Laurent Perez, IRB and Ilaria Campo and Maurizio Luisetti, Policlinico San Matteo IRCCS, Pavia, IT.

Immunogenetic mechanisms driving norovirus antigenic variation

Martina Beltramello, Blanca Fernandez-Rodriguez, Davide Corti and Antonio Lanzavecchia

Noroviruses of the GII.4 strains are the principal cause of epidemic gastroenteritis worldwide. The major capsid protein is evolving rapidly resulting in new epidemic strains with altered antigenic potentials. From immune donors we isolated a panel of human monoclonal antibodies directed against GII.4 strains and compared the reactivity of these antibodies to a panel of time-ordered GII.4 VLPs using EIAs and surrogate neutralization assays. We found a broadly cross reactive antibody that differentially blocks GII.4-1987 through 2009 VLP interaction with ligand, which represents a potential immunotherapeutic for the treatment of acute or chronic GII.4 disease. Using the antibody panel we also defined two surface exposed epitopes that evolve over time. Importantly, antigenic variation in one of these epitopes correlated with changing ligand binding patterns over time, supporting the proposed relationship between epitope escape from human herd immunity and changing target usage for virus docking and entry.

This work is done in collaboration with Lisa Lindesmith and Ralph Baric, University of North Carolina Chapel Hill, NC, US.

Long-term culture of normal and malignant plasma cells

Dora Pinto and Antonio Lanzavecchia

Plasma cells can survive in the bone marrow in a specialized niche organized by mesenchymal stromal cells. In conventional cell cultures plasma cells die rapidly, a fact that has prevented a detailed analysis. We found that bone marrow mesenchymal stromal cells (MSC) are suitable feeder cells to support the survival of human plasma cell *in vitro*. $CD138^+$ plasma cells isolated from peripheral blood or bone marrow were seeded

as single cells on MSC monolayers and IgG production was monitored over several weeks. We found that over this period, Ig accumulated in the culture supernatants at a constant rate (70-140 pg/cell/day). IgG and IgA secreting plasma cells were maintained in culture with a plating efficiency ranging from 65% to 100%, while the plating efficiency of IgM plasma cells was lower. Ig production was unaffected by hydroxyurea and irradiation, as expected for terminally differentiated non-dividing cells. We have adapted the single cell culture method to interrogate circulating plasma cells isolated seven days after infection or vaccination. The culture supernatants were screened for the presence of specific antibodies using parallel ELISA and from selected cultures the Ig genes were rescued by RT-PCR, inserted in an appropriate vector that was then transfected into 293T cells in order to produce recombinant antibodies. We have also used this method to study myeloma cells in cultures. Our findings indicate that the rate of Ig secretion is often lower than that of normal plasma cells and that drugs that target autophagy and proteasome function can synergistically inhibit plasma cell survival.

This work is done in collaboration with Francesca Fontana and Roberto Sitia, San Raffaele Scientific Institute, Milan, IT.

The analysis of newly generated plasma cells supports a dynamic model of serological memory

Dora Pinto and Antonio Lanzavecchia

Plasma cells are found in peripheral blood in the steady state and their number increases dramatically seven days after a booster immunization when a large fraction is antigen-specific.
A fraction of circulating plasma cells is CD62L$^+$ DR$^+$ and Ki67$^+$ and is therefore recently generated, while the remaining plasma cells lack these markers and are thought to represent old cells that are dislocated from the bone marrow and are bound to die. Using cultures of single CD62L$^+$ DR$^+$ plasma cells, we interrogated the repertoire of newly generated plasma cells. We found that seven days following vaccination with influenza virus, most of the recently generated plasma cells produce vaccine-specific antibodies, while a sizeable fraction produce antibodies of unknown specificities or even antibodies specific for irrelevant recall antigens. Similarly, a low frequency of recently generated plasma cells isolated in the steady state produced antibodies to vaccines or viruses that the donor had not encountered over several decades. Representative monoclonal antibodies were isolated from specific plasma cells using RT-PCR, demonstrating that in the absence of specific stimulation there is a continuous generation of plasma cells at low rate. These findings support a dynamic model of serological memory where a continuous activation of memory B cells by persisting antigen or by non-specific stimuli leads to the sustained generation of new plasma cells that compensate for those that turnover.

BCR expression and signalling in human IgA and IgM plasma cells

Dora Pinto, Antonio Lanzavecchia and David Jarrossay

Plasma cells are terminally differentiated cells of the B cell lineage that secrete antibodies at high rate and are thought to lack the expression of membrane bound immunoglobulins. Clear differences between the IgG and the IgA humoral systems in terms of dynamics have recently emerged. Here, we report that human IgA and IgM plasma cells express membrane IgA and IgM respectively. Signaling from membrane IgA and IgM leads to ERK1/2 phosphorylation and Ca^{2+} flux in an apparent Syk dependent fashion despite the absence of detectable surface Igα/Igβ heterodimer for IgA. Unlike IgM, IgA signaling induces phospho-AKT and phosphorylation of both ERK1/2 and AKT is at least partially dependent on BCR internalization. Finally, BCR cross-linking led to CD44 up-regulation and impacted survival of IgA plasma cells. These findings demonstrate fundamentally distinct biology between IgG, IgM and IgA plasma cells and suggest that the IgA plasma cell repertoire may be modulated by the presence of specific antigens.

Insights into the pathogenesis of pemphigus vulgaris from the analysis of autoantibodies

Davide Corti and Antonio Lanzavecchia

Pemphigus vulgaris is an autoimmune blistering disease of skin and mucous membranes associated with autoantibodies that bind to the cadherin-type adhesion molecules desmoglein (Dsg)3 and Dsg1, causing loss of keratinocyte cell adhesion. By immortalizing memory B cells of PV patients, we isolated 15 IgG antibodies specific for Dsg3. These antibodies used different VH and VL genes and carried high levels of somatic mutations in the CDRs of H and L chains. Three antibodies disrupted keratinocyte monolayers *in vitro* and were pathogenic in a passive transfer model in neonatal mice. The epitopes recognized by the pathogenic antibodies were mapped to the Dsg3 EC1 and EC2 subdomains in regions involved in cis-adhesive interactions. (Figure 3) Using an assay based on the capacity of patients' serum to inhibit binding of human monoclonal antibodies to Dsg3, we found that the cis-adhesive interface on EC1 recognized by the pathogenic monoclonal antibody PVA224 is the primary target of PV autoantibodies present in patients' sera. In contrast, autoantibodies that target the transadhesive interface appear to be less frequent in PV patients. These results identify the cisadhesive interface as the immunodominant region targeted by pathogenic antibodies in PV. We are currently testing the germlined version of the pathogenic antibodies to investigate the role of somatic mutation and inciting antigens in this autoimmune disease.

This work is done in collaboration with Giovanna Zambruno and Giovanni Di Zenzo, IDI-IRCCS, Roma, IT and with Fabrizia Vanzetta and Gloria Agatic, Humabs BioMed, Bellinzona, CH.

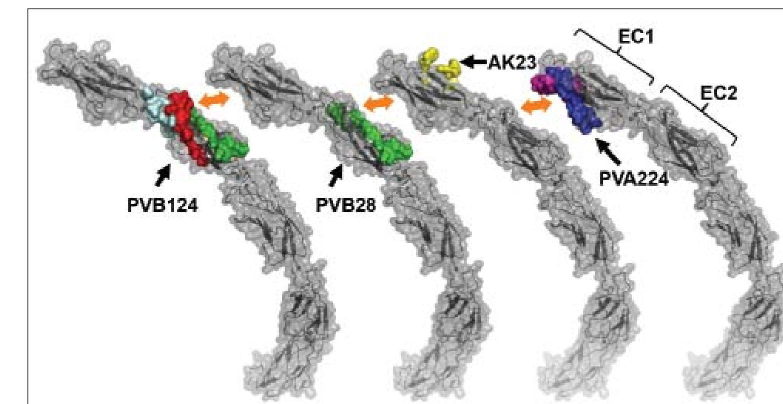

Figure 3. *Autoantibodies disrupt the cis interaction of desmoglein 3 arrays.*

Production and use of HCMV glycoprotein complexes: identification of target molecules and *in vivo* test of an HCMV subunit vaccine

Anna Kabanova, Daniele Lilleri and Antonio Lanzavecchia

To understand the cellular tropism of HCMV, identify the molecular targets and characterize the protective T and B cell response, we produced viral glycoprotein complexes in a soluble form. The gH/gL/UL128-131A pentamer complex, the gH/gL dimer and gB were produced in transfected cells, purified and shown to preserve all the conformational epitopes targeted by a panel of neutralizing antibodies. These complexes were used to identify the target molecules on epithelial cells and fibroblasts using co-precipitation and mass spectrometry analysis. The receptor for the gH/gL dimer on fibroblasts was identified and work is ongoing to find the receptor for the gH/gL/UL128-131A pentamer complex on endothelial and epithelial cells using siRNA and candidate gene transfection approaches. Glycoprotein-specific and site-specific serological assays were developed to monitor the immune response to the virus with the aim of identifying the presence of protective antibodies in serum of pregnant women. In parallel we are studying the T cell response to individual HCMV glycoproteins using the T cell library method. To develop a HCMV subunit vaccine we immunized mice with gH/gL/UL128-131A pentamer complexes and analyzed the antibody response elicited both in the serum and through the isolation of mouse monoclonal antibodies. This vaccine elicited a strong antibody response that neutralizes infection of fibroblasts as well as endothelial, epithelial and myeloid cells.

This work is done in collaboration with Federica Sallusto and Laurent Perez, IRB; Giuseppe Gerna, Fondazione IRCCS Policlinico San Matteo, Pavia, IT.

Early maternal antibody response to neutralizing epitopes on the gH/gL/pUL128-131 pentameric complex correlate with protection from vertical transmission of HCMV

Daniele Lilleri, Anna Kabanova, Davide Corti and Antonio Lanzavecchia

Human cytomegalovirus (HCMV) expresses two membrane glycoprotein complexes: a dimer of gH/gL, and a pentamer comprising gH/gL/pUL128/pUL130/pUL131, that mediate HCMV entry into fibroblasts or epithelial/endothelial cells, respectively. We characterized the serum antibody responses to these complexes in serial serum samples collected from 43 pregnant women following HCMV primary infection using both neutralization and binding assays. Total binding antibodies, as well as antibodies specific for discrete neutralization sites of the pentamer were measured using direct or competitive ELISA using recombinant complexes and monoclonal antibodies of known specificity. Neutralizing antibodies appeared early and absorption with pentamer (but not with dimer or gB) abolished the capacity to neutralize HCMV infection of epithelial cells. Antibodies to the pentamer were significantly higher than against the dimer. Furthermore, antibodies against distinct neutralization sites on the pentamer showed individual kinetics and their presence in the serum was found to correlate with protection from HCMV vertical transmission. The protective role of these antibodies *in vivo* is suggested by their *in vitro* inhibitory activity on HCMV cell-to-cell spreading and virus transfer to leukocytes. Taken together these findings indicate HCMV pentamer complex is the main target of protective antibodies.

This work is done in collaboration with Giuseppe Gerna, Fondazione IRCCS Policlinico San Matteo, Pavia, IT.

From the analysis of the human immune response to novel therapies and vaccines

Antonio Lanzavecchia and Federica Sallusto

The high throughput analysis of the human immune response to pathogens and vaccines can be used to precisely identify the specificity and class of neutralizing antibodies and protective T cells. This information can then be used to design antigens and adjuvants capable of eliciting the desired response of the same specificity and class. For B cells, we start from the analysis of the human antibody response to isolate monoclonal antibodies, which are used to study the mechanisms of protection, identify the most conserved targets and guide the design of a vaccine capable of eliciting antibodies of the same specificity. (Figure 4) For T cells, we analyse pathogen specific memory T cells to identify the antigens and epitopes recognized, the homing receptors expressed, and the cytokines produced that collectively determine the property of effector and memory T cells. We also "reconstruct" human T cell priming *in vitro* in order to dissect the signals required for T cell activation and polarization and identify the most appropriate adjuvants.

Zielinski C. et al. *
Immunol Rev. 2011, 240:40-51.

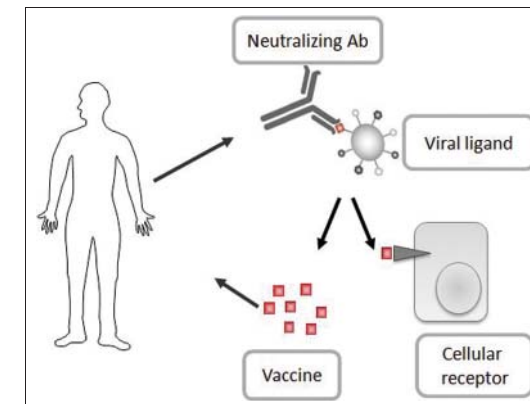

Figure 4.
The use of human monoclonal antibodies in analytic vaccinology.

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Publications

Strain-transcending Fc-dependent killing of plasmodium falciparum by merozoite surface protein 2 allele-specific human antibodies.
Stubbs, J., S. Olugbile, B. Saidou, J. Simpore, G. Corradin and A. Lanzavecchia.
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Improved innate and adaptive immunostimulation by genetically modified HIV-1 protein expressing NYVAC vectors.
Quakkelaar, E.D., A. Redeker, E.K. Haddad, A. Harari, S.M. McCaughey, T. Duhen, A. Filali-Mouhim, J.P. Goulet, N.M. Loof, F. Ossendorp, B. Perdiguero, P. Heinen, C.E. Gomez, K.V. Kibler, D.M. Koelle, R.P. Sekaly, F. Sallusto, A. Lanzavecchia, G. Pantaleo, M. Esteban, J. Tartaglia, B.L. Jacobs and C.J. Melief.
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Dissecting the human immunologic memory for pathogens.
Zielinski, C.E., D. Corti, F. Mele, D. Pinto, A. Lanzavecchia and F. Sallusto.
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An anti-HIV-1 V3 loop antibody fully protects cross-clade and elicits T-cell immunity in macaques mucosally challenged with an R5 clade C SHIV.
Watkins, J.D., N.B. Siddappa, S.K. Lakhashe, M. Humbert, A. Sholukh, G. Hemashettar, Y.L. Wong, J.K. Yoon, W. Wang, F.J. Novembre, F. Villinger, C. Ibegbu, K. Patel, D. Corti, G. Agatic, F. Vanzetta, S. Bianchi, J.L. Heeney, F. Sallusto, A. Lanzavecchia and R.M. Ruprecht.
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High throughput cellular screens to interrogate the human T and B cell repertoires.
Corti, D., F. Sallusto and A. Lanzavecchia.
Curr Opin Immunol. 2011; 23: 430-5.

In-depth analysis of the antibody response of individuals exposed to primary dengue virus infection.
de Alwis, R., M. Beltramello, W.B. Messer, S. Sukupolvi-Petty, W.M. Wahala, A. Kraus, N.P. Olivarez, Q. Pham, J. Brian, W.Y. Tsai, W.K. Wang, S. Halstead, S. Kliks, M.S. Diamond, R. Baric, A. Lanzavecchia, F. Sallusto and A.M. de Silva.
PLoS Negl Trop Dis. 2011; 5: e1188.

A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins.
Corti, D., J. Voss, S.J. Gamblin, G. Codoni, A. Macagno, D. Jarrossay, S.G. Vachieri, D. Pinna, A. Minola, F. Vanzetta, C. Silacci, B.M. Fernandez-Rodriguez, G. Agatic, S. Bianchi, I. Giacchetto-Sasselli, L. Calder, F. Sallusto, P. Collins, L.F. Haire, N. Temperton, J.P. Langedijk, J.J. Skehel and A. Lanzavecchia.
Science. 2011; 333: 850-6.

Memory in disguise.
Sallusto, F. and A. Lanzavecchia.
Nat Med. 2011; 17: 1182-3.

Characterization of neutralizing profiles in HIV-1 infected patients from whom the HJ16, HGN194 and HK20 mAbs were obtained.
Balla-Jhagjhoorsingh, S.S., B. Willems, L. Heyndrickx, K. Vereecken, W. Janssens, M.S. Seaman, D. Corti, A. Lanzavecchia, D. Davis and G. Vanham.
PLoS One. 2011; 6: e25488.

Pathogen-induced human TH17 cells produce IFN-γ or IL-10 and are regulated by IL-1β.
Zielinski C.E., F. Mele, D. Aschenbrenner, D. Jarrossay, F. Ronchi, M. Gattorno, S. Monticelli, A. Lanzavecchia, and F. Sallusto.
Nature. 2012; 484: 514-518.

Lectures and Seminars

CMCBI Educational Day and Seminar Series
Seminar "Dissecting the human memory B cell and plasma call response to pathogens and vaccines"
London (UK) / 24.02.2011

Utrecht University Eijkman Seminar Series
Seminar "Dissecting the human immune response to pathogens"
Utrecht (NL) / 02.03.2011

Meeting for Young Researchers / ESF-JSPS Frontier Science
Hulshorst (NL) / 04.03.2011

Institut Pasteur Vaccinology Course 2011
Lecture "Immunological memory: the challenge of conferring long-term protection?"
Paris (FR) / 14.03.2011

Istituto Giannina Gaslini Lecture
Lecture "Dall'analisi della memoria immunologica a nuovi vaccini e terapie"
Genoa (IT) / 15.04.2011

Università degli studi di Parma Seminar Series
Seminar "Dissecting the human T and B cell response to pathogens"
Parma (IT) / 27.04.2011

University of Zurich
Seminar "Dissecting the human antibody response to pathogens"
Zurich (CH) / 06.05.2011

BioBusiness Advanced Short Course on BioEntrepreneurship at USI
Lugano (CH) / 09.05.2011

Institut Pasteur
Seminar "The human antibody response to influenza virus"
Paris (FR) / 16.05.2011

3rd International Workshop Inflammation and Immunity
Vienna (AU) / 20.05.2011

Symposium on Primary Immunodeficiency
London (UK) / 26.05.2011

650° dell'Università di Pavia - Un futuro in ogni cortile
Pavia (IT) / 28.05.2011

EEACI 2011 Congress
Novel therapies in allergic and infectious diseases
Istanbul (TR) / 12.06.2011

The Henry Kunkel Society annual meeting
The human antibody response to influenza virus
Washington (US) / 23.06.2011

FOCIS Annual Meeting
Washington (US) / 24.06.2011

3rd Summer School of Immunology
Spetses Island (GR) / 28.06.2011

The 4th ESWI Influenza Conference
Malta, 13.09.2011

IFIR 2011: 2nd International Forum on Immunoglobulin Research
Barcelona (SP) / 16.09.2011

3rd Kitasato Symposium
Potsdam (DE) / 22.09.2011

5th Vaccine and ISV Global Congress
Seattle (US) / 02.10.2011

Seattle Biomedical Research Institute
Seminar "Dissecting the human antibody response to pathogens"
Seattle (US) / 03.10.2011

8th Nestlé International Nutrition Symposium
Lausanne (CH) / 20.10.2011

20th Congress of the European Academy of Dermatology and Venereology
Lisbon (PT) / 22.10.2011

Campus des Cordeliers
Lecture: "Dissecting the human T and B cell response to pathogens"
Paris (FR) / 04.11.2011

Karolinska Institute
Seminar: "Dissecting the human T and B cell response to pathogens"
Stockholm (SE) / 15.12.2011

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 a post-doc in the laboratory of Cesare Montecucco at the Dept. of Biomedicine, University of Padua, Italy and subsequently in the laboratory of Ari Helenius at the ETH-Zurich (1998-2000). Since October 2000, he is group leader at the IRB in Bellinzona. The studies performed by Dr. Molinari's group at the IRB significantly contributed to the knowledge of mechanisms devised by cells for the production of functional polypeptides and for efficient disposal of folding-defective proteins. The knowledge acquired on the mechanisms of protein production and transport along the secretory line of mammalian cells allowed the group to set up a novel approach based on intracellular expression of specific single chain antibodies that proved very efficient in reducing the *in vivo* production of amyloid-beta (Aβ), a toxic peptide that deposits in the human brain eliciting neurodegenerative processes associated with the Alzheimer's disease. Dr. Molinari received the Science Award 2002 from the Foundation for the study of neurodegenerative diseases, the Kiwanis Club Award 2002 for Medical Science, the Friedrich-Miescher Award 2006 and the Research Award Aetas 2007. Since 2008, Dr. Molinari is Adjunct Professor at the ETH-Lausanne.

Research Focus

The endoplasmic reticulum (ER) contains high concentrations of molecular chaperones and enzymes that assist maturation of newly synthesized polypeptides destined to the extracellular space, the plasma membrane and the organelles of the endocytic and secretory pathways. It also contains quality control factors that select folding-defective proteins for ER retention and/or ER-associated degradation (ERAD). Mutations, deletions and truncations in the polypeptide sequences may cause protein-misfolding diseases characterized by a "loss-of-function" upon degradation of the mutant protein or by a "gain-of-toxic-function" upon its aggregation/deposition. Pathogens hijack the machineries regulating protein biogenesis, quality control and transport for host invasion, genome replication and progeny production. The aim of our work is to understand the molecular mechanisms regulating chaperone-assisted protein folding and the quality control processes determining whether a polypeptide can be secreted, should be retained in the ER, or should be transported across the ER membrane for degradation. A thorough knowledge of these processes will be instrumental to design therapies or to identify drug targets for interventions aiming at delaying the progression or even at curing diseases caused by inefficient functioning of the cellular protein factory, resulting from expression of defective gene products, or elicited by pathogens.

Team

Group Leader: Maurizio Molinari, PhD > maurizio.molinari@irb.usi.ch
Members: Riccardo Bernasconi, PhD – Elisa Fasana, PhD - Carmela Galli Molinari, MSc - Jessica Merulla, PhD student - Julia Noack, PhD student -Tatiana Soldà, MSc

Substrate-specific mechanisms of protein degradation from the ER

Jessica Merulla, Tatiana Soldà, Elisa Fasana and Maurizio Molinari

Misfolded polypeptides produced in the ER are dislocated across the ER membrane to be degraded by cytosolic 26S-proteasomes in processes collectively termed ER-associated degradation (ERAD). Dislocation across the ER membrane is regulated by multimeric complexes built around one of the several membrane-embedded E3 ubiquitin ligases expressed in the mammalian ER (e.g. HRD1, gp78, TEB4, Trc8, RFP2, RMA1). It is postulated that specific physico-chemical features of the misfolded polypeptide (e.g. presence/absence of N-linked oligosaccharides, disulfide bonds, peptidyl-prolyl bonds in the *cis* conformation, membrane-anchor) determine the quality control machineries that deliver the misfolded polypeptide at specific dislocation complexes. The definition of the rules that govern protein biogenesis and quality control requires a systematic analysis of the behavior of appositely designed model folding-competent and folding-defective proteins. We have therefore prepared more than 50 model substrates with select physico-chemical features (e.g. with/without membrane anchors, destabilizing charged residues in the transmembrane domains, oligosaccharides, *cis* prolines, cysteines). We are planning i) to perform comparative studies of the behavior of these model proteins transiently or stably expressed in mammalian cells; ii) to systematically analyze the consequences of silencing of known ERAD factors expression on quality control of the select model polypeptides; iii) to use these model polypeptides as baits to co-immunoisolate interacting cellular partners possibly engaged to assist maturation, quality control, secretion or retention in the ER, selection for degradation, extraction from the folding machinery, dislocation across the ER membrane, poly-ubiquitylation, proteolytic degradation. So far, our studies demonstrated the crucial role of N-linked oligosaccharides processing and recognition in protein quality control (reviewed in Aebi et al TIBS 2010). We also demonstrated that disposal of Soluble (non-transmembrane) polypeptides with luminal lesions (ERAD-LS substrates) is strictly dependent on the E3 ubiquitin ligase HRD1, the associated cargo receptor SEL1L and two interchangeable ERAD lectins, OS-9 and XTP3-B. These ERAD factors become dispensable for degradation of the same polypeptides when Membrane-tethered (ERAD-L_M substrates) (Bernasconi et al 2010, Figure 1).

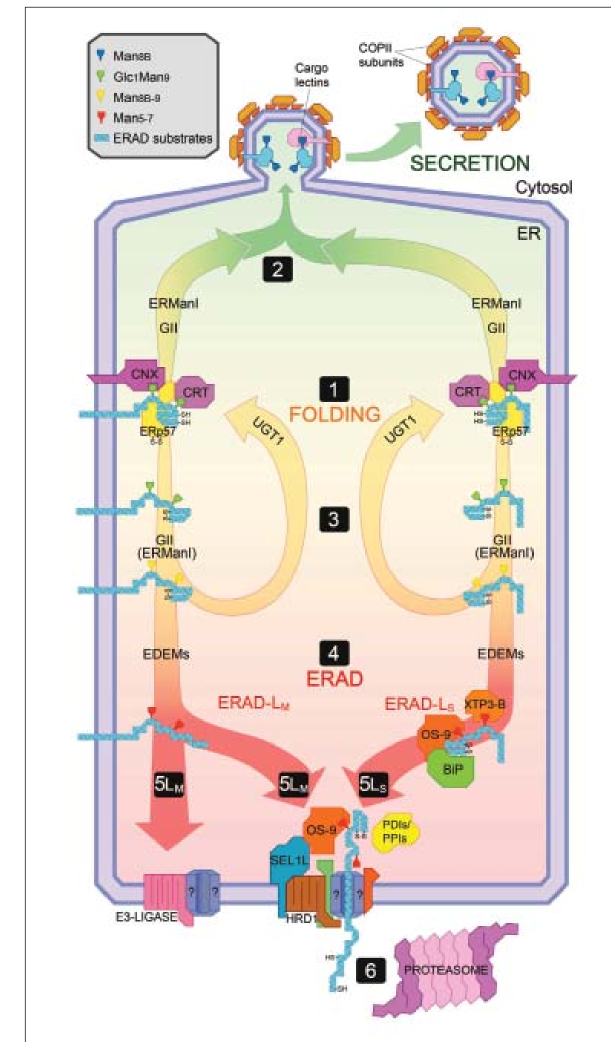

Figure 1

Folding and ERAD pathways in the mammalian ER lumen. CNX, CRT and the oxidoreductase ERp57 assist formation of native disulfide bonds (step 1). Native glycopolypeptides are secreted in coat protein complex II (COPII)-coated vesicles and are transported at their final destination (step 2). Non-native glycopolypeptides are retained in the CNX chaperone system by the UGT1 that adds-back one terminal glucose residue (step 3). Extensive de-mannosylation irreversibly extracts terminally misfolded polypeptides from the CNX cycle (step 4). Misfolded proteins engage multiple pathways to be directed at and be eventually dislocated across the ER membrane (steps 5). Degradation is carried out by 26S-proteasomes (step 6).

Comparative interactomic to identify novel ER-resident quality control players

Jessica Merulla, Tatiana Soldà, Carmela Galli Molinari and Maurizio Molinari

The aim of the project is to identify new players that intervene in protein folding, quality control and ERAD in the mammalian ER lumen. We are preparing a series of stable human cell lines expressing epitope-tagged folding-competent and folding-defective proteins. The model proteins are used as baits to capture interacting partners in the same immuno-complexes. The proteins co-immuno-isolated with the individual baits are subjected to tryptic digestion and fragments are separated by nano-HPLC followed by tandem MS. Fragmentation spectra of the samples are matched to a human protein database sequence with the Mascot software. These analytic steps are performed in collaboration with Manfredo Quadroni, Coordinator of the Center for Integrative Genomics, University of Lausanne. Involvement of the model protein's interacting partners in protein quality control will be validated upon their down regulation by specific RNA interference. Preliminary experiments have been performed to compare the interacting partners of BACE501, a secretory protein, with the interacting partners of BACE476, a folding-defective deletion mutant of BACE501. ER-resident proteins specifically interacting with BACE476 are thought to be involved in retention and disposal of this folding-defective polypeptide since they ignore the folding competent BACE501 (Figure 2A). In another example, the comparative interactome analysis of a misfolded module (NHK) anchored at the membrane with a conventional (BACE) or with a destabilized (CD3-δ) transmembrane domain (Figure 2B) hints at possible engagement of specific quality control factors (black arrows, unpublished) by membrane domains with charged residues. Their involvement in quality control of transmembrane regions will be confirmed experimentally (e.g. silencing of a candidate regulatory factor should differently affect disposal kinetics of the model-substrates-under-investigation).

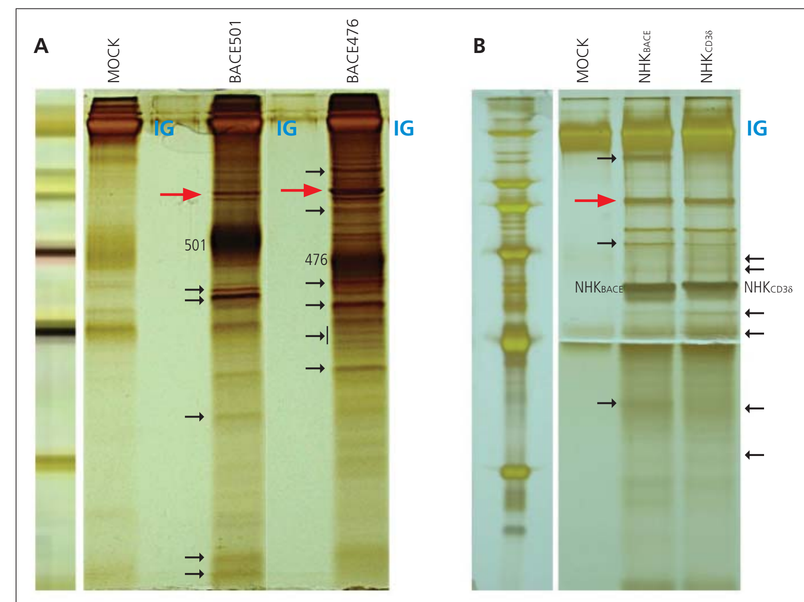

Figure 2
Interactomic studies.
A, IG shows the position of the anti-HA used for immunoisolation of complexes containing the folding competent BACE501 or the folding-defective BACE476.
The red arrows show CNX, black arrows show relevant interacting proteins, which have been identified by tryptic cleavage/nano HPLC/ tandem MS. B, same as A for the two folding defective NHK_{BACE} and NHK_{CD38}. Gels have been silver stained.

Disposal of non-glycosylated polypeptides from the mammalian ER

Elisa Fasana, Jessica Merulla, Tatiana Soldà and Maurizio Molinari

To maintain ER homeostasis and to ensure the highest efficiency of functional polypeptide production, the quality control machinery operating in the mammalian ER must distinguish non-native intermediates of protein folding programs from terminally misfolded polypeptides. Folding intermediates must be retained in the ER to attain the native structure under the assistance of dedicated molecular chaperones and folding enzymes. Terminally misfolded polypeptides must be, on the other hand, rapidly cleared from the ER lumen to avoid interferences with ongoing folding programs. For glycosylated polypeptides, which represent the majority of the cargo entering the secretory pathway, the processing of the N-linked oligosaccharides determines retention in the folding environment (cycles of removal/re-addition of terminal glucose residues) or extraction from the folding environment for disposal (removal of terminal mannose residues, reviewed in Aebi et al. TIBS 2010). Virtually nothing is known about quality control of non-glycosylated polypeptides. We have generated a series of glycosylation mutants of the model glycopolypeptides studied in our lab and we will monitor their behavior in wild type cells and in cells lacking or expressing low levels of select ER-resident quality control factors. The aim of this project is the identification of ER-resident factors involved in quality control and disposal of non-glycosylated polypeptides.

ERAD tuning: The selective clearance of ERAD regulators from the ER lumen

Riccardo Bernasconi, Julia Noack, Carmela Galli Molinari and Maurizio Molinari

Several regulators of ERAD have shorter half-life compared to conventional ER chaperones. At steady state, they are selectively removed from the ER in a series of poorly defined events that we named ERAD tuning. In an environment, the ER, where folding and disposal of newly synthesized cargo polypeptides are in kinetic competition, ERAD tuning sets ERAD activity at levels that do not interfere with completion of ongoing folding programs and is therefore crucial to maintain cellular proteostasis. We have identified the complex comprising the type-I ER protein SEL1L and the cytosolic protein LC3-I as an ERAD tuning receptor that regulates the COPII-independent vesicle-mediated removal of the luminal ERAD regulators EDEM1 and OS-9 from the ER (Figure 3A). Luminal expression of folding-defective polypeptides enhances the content of EDEM1 and OS-9 by inhibiting their SEL1L:LC3-I-mediated clearance from the ER thereby selectively rising ERAD activity in the absence of induction of transcriptional programs known as unfolded protein responses (UPR) (Figure 3B). The aim of this project is to identify the chaperones/enzymes whose intraluminal level is regulated by ERAD tuning, and to characterize the mechanisms regulating their segregation from long-lived chaperones that are retained in the bulk ER. Since the vesicular export of select ERAD factors from the ER is hijacked by pathogens (see next project), the characterization of the mechanisms regulating ERAD tuning and the identification of the cellular proteins involved in this process might lead to the identification of potential targets for anti-viral therapies.

* ***Bernasconi, R. and Molinari, M.***
Curr Op Cell Biol 2011, 23:176-183.

ERAD tuning: Hijacking by viral pathogens

Riccardo Bernasconi and Maurizio Molinari

We have established that Coronaviruses (CoV) hijack the host cell ERAD tuning machinery during their infection cycle. In fact, the mouse hepatitis virus (MHV), a prototype CoV, co-opts the ER-derived vesicles containing EDEM1, OS-9, SEL1L and LC3-I, the EDEMosomes, and uses them as a scaffold to build viral replication and transcription complexes. MHV replication is significantly impaired upon silencing of SEL1L and LC3, which are required for EDEM1 and OS-9 segregation from the ER. Our data highlight the biological relevance of a novel COPII-independent ER export pathway, which is hijacked by mammalian pathogens. Before our reports (Calì et al 2008 and Reggiori et al 2010), LC3-I was simply considered as a cytosolic precursor of the autophagosomal protein LC3-II. By revealing the role of LC3-I in ERAD tuning and in cell infection by CoV and by Arteriviruses, our studies show for the first time an autophagy-independent function of this ubiquitin-like protein.

* ***Reggiori F. et al.***
Viruses 2011, 3:1610-1623.

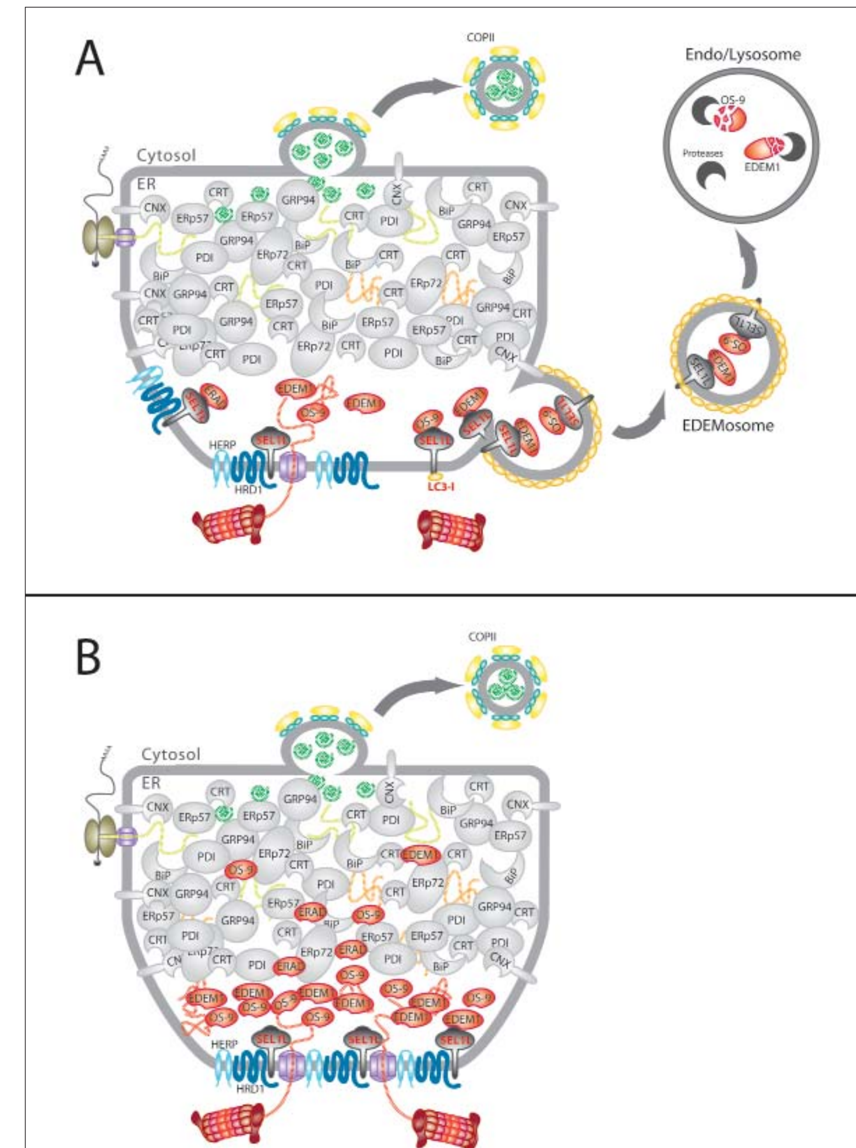

Figure 3
A, At steady state, ERAD regulators such as EDEM1 and OS-9 are selectively exported from the ER. Export is receptor (SEL1L:LC3-I)-mediated. The ER-derived EDEMosomes are hijacked by CoV for replication.
B, Misfolded polypeptides prevent association of EDEM1 and OS-9 with the SEL1L:LC3-I receptor that regulates their clearance from the ER. This results in an UPR-independent enhancement of the intraluminal concentration of these ERAD regulators.

Mechanisms regulating the recovery from acute ER stress in mammalian cells

Julia Noack, Riccardo Bernasconi and Maurizio Molinari

Expression of misfolded polypeptides, variations in the ER environment or in the ER cargo load may result in induction of unfolded protein responses (UPR). A series of stress sensors transduce the stress signal across the ER membrane thereby causing a global inhibition of transcription and translation, the selective up-regulation of folding and ERAD factors and the swelling of the ER upon enhanced lipid-biogenesis. The aim of this project is to establish how cells return to the "steady state situation" after having experienced a phase of acute stress that resulted in the transient enhancement of folding and ERAD factors concentration in the ER lumen. In particular, we will assess how cells re-establish the steady state intraluminal concentrations of molecular chaperones and enzymes during the recovery phase. Preliminary data show that ERAD factors return to their initial level faster than folding factors. This possibly indicates that ERAD tuning (i.e. the fast and selective ERAD factors clearance from the ER) plays a regulatory role during recovery from ER stress. Since autophagy is essential for maintaining the ER homeostasis in yeast and to promote survival of mammalian cells during ER stress, we will also investigate the role of autophagy or autophagy-like mechanisms in the recovery phase.

Characterization of Malectin, a novel ER-resident lectin

Carmela Galli Molinari and Maurizio Molinari

In the ER lumen, asparagine residues in nascent polypeptide chains are modified with a pre-assembled $Glc_3Man_9GlcNAc_2$- oligosaccharide. The highly hydrophilic oligosaccharide increases the solubility of unstructured nascent chains. In a matter of seconds, the two terminal glucose residues are removed, generating a protein-bound mono-glucosylated oligosaccharide ($Glc_1Man_9GlcNAc_2$-) that recruits the lectin chaperones calnexin (CNX) and calreticulin (CRT). Substrate association with the lectin chaperones facilitates attainment of the native structure, a prerequisite for release of newly synthesized polypeptides from the ER and transport along the secretory pathway to their final destination. Recently, the existence of Malectin in the Metazoan proteome has been reported. Among almost 300 structures examined by carbohydrate microarray, the Xenopus protein showed a unique selectivity of binding to a di-glucosylated high-mannose N-glycan sequence. Since current models claim that di-glucosylated oligosaccharides are only very transiently displayed on newly synthesized polypeptides and are therefore unlikely to play significant roles in protein quality control in the ER, the investigation into the function of Malectin in the mammalian ER is of interest. Our studies showed that Malectin is an ER stress-induced protein. It selectively associates with glycopolypeptides without affecting their entry and their retention in the CNX chaperone system. Analysis of the *obligate* CNX client influenza virus hemagglutinin (HA) revealed that CNX and Malectin associated with different HA conformers at different times and that Malectin preferentially associated with misfolded HA. Analysis of the *facultative* CNX clients NHK and α1AT revealed that induction of Malectin expression, to simulate conditions of ER stress, resulted in persistent association between the ER lectin and the model cargo glycoproteins, interfered with processing of cargo-linked oligosaccharides and reduced cargo secretion. We propose that Malectin intervention is activated upon ER stress to inhibit secretion of defective gene products that might be generated upon aberrant functioning of the ER quality control machinery.

* ***Galli C. et al.*** *PLoS ONE 2011, 6:e16304.*

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

Siro Bianchi, Carmela Galli Molinari and Maurizio Molinari

Sequential cleavages of the human amyloid precursor protein (APP) by beta- and gamma-secretases generate the amyloid-beta (Aβ), a 42-residues, aggregation-prone toxic peptide associated with neurodegeneration in Alzheimer's disease (AD). We have generated vectors for expression in mammalian cells of single chain antibodies, Fab fragments and full length monoclonal antibodies specifically binding residues 3 to 6 of the Aβ peptide. When expressed intracellularly, these molecules associate with newly synthesized APP, thus substantially interfering with beta-secretase cleavage and Aβ production. When added extracellularly, they associate with surface exposed APP similarly interfering with Aβ production (Paganetti et al 2005).
Therapeutic monoclonal antibodies (mAbs) constitute a promising avenue for the treatment of several major diseases including autoimmune, cardiovascular, neurodegenerative, infectious diseases, cancer and inflammation. Major drawbacks that presently limit the use of therapeutic antibodies following systemic delivery are related to the poor distribution at the targeted tissues, inadequate pharmacokinetics, and elevated costs of manufacture. We propose a novel way to potentially release mAbs or antibody fragments in targeted tissues for extended periods of time using semi-permeable polymeric cell implants. Surrounding genetically engineered C2C12 cells producing mAbs and/or antibody fragments with a synthetic permoselective membrane minimizes immunological responses by avoiding cell-to-cell contact between the host tissue and the encapsulated cells. The permeable membrane of the implants allows the inward diffusion of nutrients and oxygen and the outward diffusion of antibodies into the implanted tissue. As proof-of-concept, we tested this technology as an immunotherapeutic approach for the treatment of AD using a transgenic mouse model of the disease. Implants of cells releasing single-chain fragment variable (scFv) antibodies placed in the brain parenchyma of APP23 transgenic mice proved to be capable of continuously processing, expressing and secreting the scFvβ1 antibody fragment targeted against the EFRH epitope of the Aβ peptide. *In situ* chronic expression of scFvβ1 following a six-month immunotherapy in 14-month old APP23 mice reduced the accumulation and production of Aβ, as analyzed with histological and biochemical markers. Functional assessment in mice showed significant behavioral recovery of anxiety and memory traits. This novel technique to deliver antibodies into targeted tissues can serve as an alternative approach for the treatment of AD and potentially other major diseases treated by passive vaccination strategies.

Marroquin* Belaunzaran O. et al. *PLoS ONE 2011, 6:e18268.*

Involvement of folding/quality control and ERAD factors in APP processing and Aβ production

Elisa Fasana, Riccardo Bernasconi and Maurizio Molinari

We have a long-standing interest in the development of reagents that interfere with the generation and deposition of the toxic Aβ peptide, a hallmark of AD (e.g. Paganetti et al JCB 2005, Marroquin et al PLoS ONE 2011). Thus, we routinely test all the cellular components identified in our experiments as folding/quality control/ERAD factors for their possible involvement in maturation and/or trafficking and/or processing of APP and of BACE501 (the aspartic protease that sheds the APP ectodomain as the initial proteolytic event leading to the Aβ production). Figure 4 shows one of these tests in which several target genes were silenced to determine their involvement in ERAD. Analysis of APP maturation revealed that silencing of one of the genes-under-investigation (the ER lectin LECT2), dramatically interfered with APP maturation and/or processing as shown by the persistency of the radiolabeled APP after 120 min of chase (Figure 4B, siLECT2 to be compared with the disappearance of the APP in all other samples (Figure 4). The involvement of LECT2 in APP maturation and/or processing was confirmed by using ectopically expressed APPsw and by the finding that LECT2 silencing dramatically reduced production of Aβ (Figure 4C).

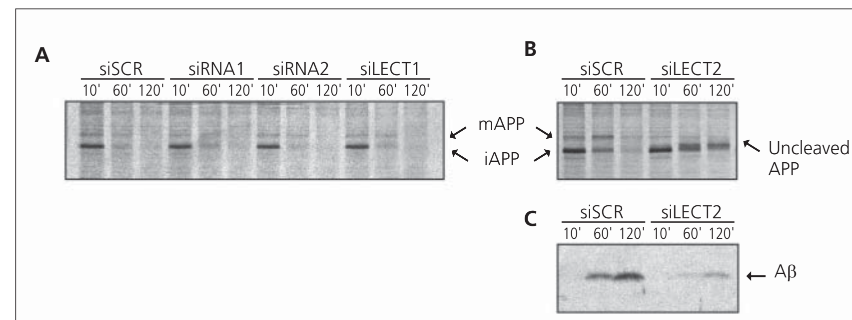

Figure 4
A Consequences on APP maturation of mock-treatment (siSCR) or of down-regulation of protein 1 (siRNA1, for small interfering RNA to protein 1), protein 2 (siRNA2) and LECT1 (siLECT1). B, same as A for LECT2 (a LECT1 homolog). C, Analysis of Aβ production in mock treated cells (siSCR) and in cells characterized by low expression of LECT2 (siLECT2).

Funding

Swiss National Science Foundation
Protein folding, quality control and degradation in the ER
3100A0-121926/2002-2011

Foundation for Research on Neurodegenerative Diseases
β-secretase as model to investigate the mechanisms of ERAD
2002-2014

Novartis Stiftung für Medizinisch Biologische Forschung
ERAD tuning
2012

S. Salvatore Foundation
Functional characterization of endoplasmic reticulum-associated protein degradation regulators implicated in tumor progression
2005-2012

Gabriele Trust
Biogenesis of proteins involved in the Alzheimer's disease
2012-2014

Association Française contre les Myopathies
Processing of disease-causing sarcoglycan mutant
MNM2Physiopath
2010-2011

Collaborations

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Lars Ellgaard
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Visiting Scientists

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January-April 2011
University of Chile, Santiago (CL)

Mirjam Hunziker
February-June 2011
ETH-Zurich (CH)

Sarah Motta
January-February 2012
University of Zurich (CH)

Publications

ERAD and ERAD tuning: disposal of cargo and of ERAD regulators from the mammalian ER.
Bernasconi, R. and M. Molinari.
Curr Opin Cell Biol. 2011, 23:176-183.

Malectin Participates in a Backup Glycoprotein Quality Control Pathway in the Mammalian ER.
Galli, C., R. Bernasconi, T. Solda, V. Calanca and M. Molinari.
PLoS One. 2011; 6:e16304.

Chronic delivery of antibody fragments using immunoisolated cell implants as a passive vaccination tool.
Marroquin Belaunzaran, O., M.I. Cordero, V. Setola, S. Bianchi, C. Galli, N. Bouche, V. Mlynarik, R. Gruetter, C. Sandi, J.C. Bensadoun, M. Molinari and P. Aebischer.
PLoS One. 2011; 6:e18268.

Unconventional Use of LC3 by Coronaviruses through the Alleged Subversion of the ERAD Tuning Pathway.
Reggiori, F., C.A. de Haan and M. Molinari.
Viruses. 2011; 3:1610-23.

Endoplasmic Reticulum-Associated Protein Degradation.
Bernasconi, R. and Molinari, M.
In Encyclopedia of Biological Chemistry. Lennarz, W.J. and Lane, M.D. Eds, Elsevier, Oxford, 2012; 1:20-23.

Lectures and Seminars

Giornate Autogestite Liceo di Bellinzona
La ricerca biomedica in Ticino
Bellinzona (CH) / 30.03.2011

2011 ICGEB Seminar Series
ERAD and ERAD tuning: disposal of cargo and of ERAD regulators from the mammalian ER
Trieste (IT) / 6-7.04.2011

The Walter Mackenzie Talk, University of Alberta
ERAD and ERAD tuning: disposal of cargo and of ERAD regulators from the mammalian ER
Edmonton (CA) / 13-16.04.2011

The ER and Redox Club Meeting 2011
ERAD and ERAD tuning: disposal of cargo and of ERAD regulators from the mammalian ER
Hohenkammer (DE) / 28-30.04.2011

UCD Conway Lectures and Seminar Series, University College Dublin
N-linked glycans in the ER: a signal for misfolded proteins degradation, a signal for chaperones turnover
Dublin (IE) / 2-4.05.2011

The Biology of Molecular Chaperones
ERAD tuning: selective and rapid disposal of ERAD regulators from the mammalian ER (Riccardo Bernasconi)
Grundlsee (AT) / 19-24.05.2011

21st International Symposium on Glycoconjugates 2011
Role of N-linked oligosaccharides in protein quality control and in maintenance of ER homeostasis
Keynote Lecture
Vienna (AT) / 21-26.08.2011

Basic Virology Course, Institut Pasteur
From protein folding to … how coronaviruses hijack the host cell ERAD tuning machinery for replication
Paris (FR) / 2.09. 2011

Quality Control: Folding and Degradation of Proteins in the Endoplasmic Reticulum 2011
ERAD Tuning: How ERAD regulators are selectively cleared from the ER at steady state to maintain proteostasis
Ascona (CH) / 11-16.09.2011

Giornata Mondiale Dedicata all'Alzheimer, Università della Svizzera Italiana
La Malattia di Alzheimer
Lugano (CH) / 21.09.2011

Engineers Shape our Future 2011, Il Mondo delle Tecnologie
La Malattia di Alzheimer
Lugano (CH) / 21-25.11.2011

Scuole Elementari, Bellinzona
La Ricerca Biomedica in Ticino
Bellinzona (CH) / 25.11.2011

Organization of international congresses

Quality Control: Folding and degradation of proteins in the Endoplasmic Reticulum 2011
(http://qc2011.ethz.ch/)
Ascona (CH) / 11-16.09.2011

Silvia Monticelli

Molecular Immunology

Silvia Monticelli

Silvia Monticelli earned her Ph.D. degree at the University of Milan (IT). She began her research training at the San Raffaele Scientific Institute in Milan (IT), where her scientific interest was sparked by the study of molecular mechanisms underlying immunological processes. After spending some time at the Randall Institute, King's College London (UK), she joined the lab of Prof. Rao at Harvard Medical School in Boston (US), where she continued her scientific training by performing studies aimed to understand the mechanisms of regulation of cytokine transcription in T lymphocytes and mast cells. In February 2007 she joined the Institute for Research in Biomedicine in Bellinzona as Group Leader. Dr. Monticelli has published several papers covering various aspects of immunological processes, with a special focus on diseases such allergy and asthma as well as mastocytosis. 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

Mastocytosis is a tumor characterized by the abnormal proliferation and accumulation of aberrant mast cells of the immune system. This disease shows a clinical course variably ranging from asymptomatic for years to highly aggressive and rapidly devastating. Although some genetic alterations at the base of mastocytosis have been described, little is known concerning pathogenetic factors that contribute to the development of disease variants and disease progression. When mastocytosis develops into aggressive forms, its clinical course can be very rapid and often fatal, hence the importance of uncovering new molecular mechanisms at the base of the development of disease variants, as well as of identifying new molecular markers for diagnosis and prognosis. Our lab is interested in understanding new genetic and epigenetic mechanisms of regulation of gene expression that might be important for the development of mastocytosis or its variants. Among these are microRNAs (miRNAs), a family of small non-coding RNAs that have emerged as key post-transcriptional regulators in a wide variety of organisms and biological processes. 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. Accordingly, genetic ablation of the miRNA machinery, as well as loss or dysregulation of certain individual miRNAs, severely compromises immune development and leads to immune disorders such as autoimmunity and cancer. In our lab we are studying the role of miRNAs in the differentiation and function 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 underlying these processes have substantial potential for clinical application in the treatment of asthma, allergy, autoimmunity, chronic inflammation and malignancies.

Team

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Transcription factor and microRNA-mediated regulation of mast cell survival.

Nicole Rusca, Lorenzo Deho' and Silvia Monticelli

Mice that lack the transcription factor NF-κB1 are unable to mount eosinophilic airway inflammation due to impaired differentiation of lymphocytes to the Th2 subset, and consequently reduced expression of the cytokines IL-4, IL-5 and IL-13. Mast cells are important effector cells in asthma and allergic diseases and can produce high amounts of Th2-type cytokines. We therefore investigated whether a defect in mast cell differentiation and/or function could contribute to the lack of airway inflammation observed in these mice.

We found that bone marrow-derived mast cells (BMMCs) from NF-κB1-deficient mice showed a strong resistance to apoptosis in response to withdrawal of essential cytokines. By further investigating the role of NF-κB1 in influencing mast cell survival, we found that BMMCs lacking NF-κB1 were severely impaired in their ability to induce expression of miR-146a, and that this miRNA plays an important role in modulating mast cell survival. Indeed, forced miR-146a expression in primary BMMCs led to an increase in cell death, pointing towards a molecular network involving both NF-κB1 and miR-146a in regulating cell survival in mast cells. Moreover, miR-146a upregulation contributed to negatively regulate NF-κB activation through the down-modulation of the signalling molecules IRAK1 and TRAF6.

While the asthma-resistant phenotype of NF-κB1-deficient animals remains primarily Th2-dependent, we identified a novel molecular network that regulates mast cell survival and homeostasis in the tissues, which could be important in diseases related to abnormal accumulation of mast cells such as mastocytosis.

* *Rusca N et al.*
Mol Biol Int. 2011; 2011:437301.

MiR-221 influences effector functions and actin cytoskeleton in mast cells

Ramon J Mayoral, Lorenzo Deho', Nicole Rusca and Silvia Monticelli

Mayoral R. et al. *
PLoS ONE 2011;6:e26133

Mast cells have essential effector and immunoregulatory functions in IgE-associated allergic disorders and certain innate and adaptive immune responses, but the role of miRNAs in regulating mast cell functions is almost completely unexplored. To examine the role of the activation-induced miRNA miR-221 in mouse mast cells, we developed robust lentiviral systems for miRNA overexpression and depletion. While miR-221 favored mast cell adhesion and migration towards SCF or antigen in trans-well migration assays, as well as cytokine production and degranulation in response to IgE-antigen complexes, neither miR-221 overexpression, nor its ablation, interfered with mast cell differentiation. Transcriptional profiling of miR-221-overexpressing mast cells revealed modulation of many transcripts, including several associated with the cytoskeleton; indeed, miR-221 overexpression was associated with reproducible increases in cortical actin in mast cells, and with altered cellular shape and cell cycle in murine fibroblasts. Our bioinformatics analysis showed that this effect was likely mediated by the composite effect of miR-221 on many primary and secondary targets in resting cells. Indeed, miR-221-induced cellular alterations could not be recapitulated by knockdown of one of the major targets of miR-221. We propose a model in which miR-221 has two different roles in mast cells: in resting cells, basal levels of miR-221 contribute to the regulation of the cell cycle and cytoskeleton, a general mechanism probably common to other miR-221-expressing cell types, such as fibroblasts. Vice versa, upon induction in response to mast cell stimulation, miR-221 effects are mast cell-specific and activation-dependent, contributing to the regulation of degranulation, cytokine production and cell adherence (Figure 1). Our studies provide new insights into the roles of miR-221 in mast cell biology, and identify novel mechanisms that may contribute to mast cell-related pathological conditions, such as asthma, allergy and mastocytosis.

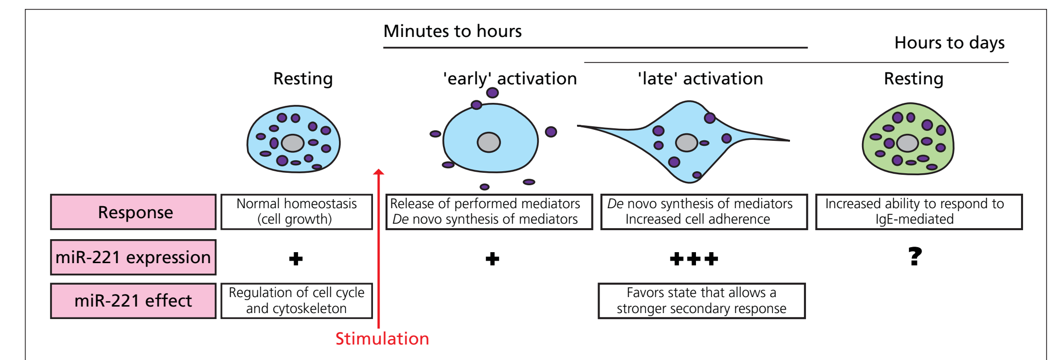

Figure 1. A 'dual' role for miR-221 in mast cells.
Working model of the possible roles of miR-221 in mast cells. At resting state, basal levels of miR-221 expression would regulate homeostatic mechanisms such as the cell cycle and cytoskeleton. These effects are not necessarily cell type-specific, as they can be active also in fibroblasts, which also express miR-221. Upon mast cell activation, 'early' effects include the release of preformed mediators from the cytoplasmic granules and the de novo synthesis of other mediators, including a broad panel of cytokines. The peak of accumulation of mature miR-221 is instead a 'late' event upon cell stimulation, and we speculate that it may contribute to the strength of the response upon secondary challenge, with increased degranulation, cytokine production and cell adherence.

Identification of novel genetic and epigenetic determinants for oncogenic transformation in patients with systemic mastocytosis

Lorenzo Debo', Sara Montagner, Nicole Rusca and Silvia Monticelli

The prognosis for systemic mastocytosis is highly dependent on the type: although indolent mastocytosis is generally not fatal, other forms (e.g. mast cell leukemia and aggressive mastocytosis) usually have a rapidly devastating outcome. While mast cells are normally present in tissues exposed to the environment like mucosal tissues in the gut, in patients with systemic mastocytosis other organs, and most notably the bone marrow, become abnormally invaded by pathogenic mast cells (Figure 2). Despite that, the pathogenetic factors that contribute to mastocytosis variants are still largely obscure. For example, mastocytosis may occur also in absence of mutations in the oncogene *KIT*, indicating that the molecular reasons behind this disease is variable, it does not depend on a single mutation or oncoprotein, and it could have both genetic and epigenetic basis. We are investigating new genetic mutations in *KIT*, as well as in newly described oncogenes, that may lead to epigenetic modifications of gene expression (specifically, alterations of miRNA regulation and abnormal patters of DNA methylation) and ultimately to tumorigenic transformation.

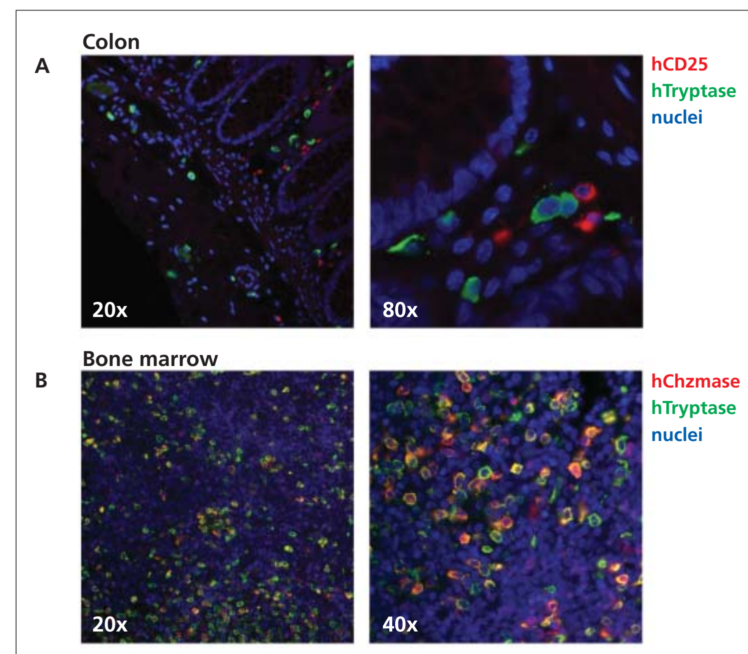

Figure 2: Immunofluorescence of paraffin-embedded sections of normal and diseased human tissues.
(A) Immunofluorescence of a paraffin-embedded section of normal human colon. Tissue section were stained for CD25 (red) and tryptase (a mast cell protease, green). Nuclei were counterstained with DAPI (blue), and images were taken with a Nikon Eclipse E800 microscope and analyzed with the Openlab software. (B) Immunofluorescence for tryptase and chymase (both mast cell proteases) of a paraffin-embedded section of a bone marrow biopsy from a patient with diagnosed systemic mastocytosis, showing a high number of pathogenic mast cells in the bone marrow. Yellow cells express both kinds of proteases.

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Collaborations

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Publications

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Pathogen-induced human TH17 cells produce IFN-γ or IL-10 and are regulated by IL-1β.
Zielinski C.E., F. Mele, D. Aschenbrenner, D. Jarrossay, F. Ronchi, M. Gattorno, S. Monticelli, A. Lanzavecchia, and F. Sallusto.
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"Mastocitosi: una, nessuna, centomila"
Siena (IT) / 24.06.2011

University of Turin Medical School
"Molecular mechanisms underlying mast cell biology"
Turin (IT) / 28.07.2011

Federica Sallusto

Cellular Immunology

Federica Sallusto

Federica Sallusto received the degree of Doctor in Biology from the University of Rome and performed two years of postdoctoral training at the Basel Institute for Immunology working in the laboratory of Antonio Lanzavecchia on human monocyte-derived dendritic cells. In 1995, she started her own group at the Istituto Superiore di Sanità in Rome focusing on the T cell response to allergens and in 1997 she became member of the Basel Institute where she started a new line of research on T cell trafficking. Her studies revealed a differential expression of chemokine receptors in human Th1 and Th2 cells and led to the characterization of “central memory” and “effector memory” T cells as memory subsets with distinct migratory capacity and effector function. Since 2000, she is a group leader at the IRB. Among her recent contributions are the characterization of human Th17 and Th22 cells and of the mechanisms that control cytokine gene expression in human T cells. Recent studies from her lab in the mouse model challenged current dogmas as to the mechanisms that control lymphocyte migration in inflamed lymph nodes and in the non inflamed central nervous system. For her scientific achievements, Federica Sallusto received the Pharmacia Allergy Research Foundation Award in 1999, the Behring Lecture Prize in 2009, and the Science Award from the Foundation for Studies of Neurodegenerative Diseases in 2010. She was elected member of the German Academy of Science Leopoldina in 2009 and member of EMBO in 2011. She is President elect of the Swiss Society for Allergology and Immunology for the period 2011-2013.

Research Focus

Our work is focused on the understanding of the mechanisms that control T cell priming and regulate cytokine production and homing capacities. These questions are addressed primarily in the human system, where we combine the *ex vivo* analysis of memory T cell subsets identified according to the expression of several markers, in particular chemokine receptors, with *in vitro* priming experiments that dissect the mechanisms of T cell differentiation. This approach has led to the identification of chemokine receptors expressed in human Th17 and Th22 cells, to the dissection of the cytokines that drive polarization and modulate effector functions of human T cells. In parallel, we have used the mouse system to address fundamental questions on the regulation of lymphocyte trafficking during inflammation and in autoimmunity. We also developed a method for the analysis of human naive and memory CD4 and CD8 T cell repertoires based on high throughput cellular screenings of human T cell libraries. This method is currently used to dissect the human T cell response to pathogens, allergens, and self-antigens.

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Pathogen-induced human Th17 cells produce IFN-γ or IL-10 and are regulated by IL-1β

Christina E. Zielinski, Federico Mele, Dominik Aschebrenner, Francesca Ronchi and Federica Sallusto

IL-17-producing CD4⁺ T helper cells (Th17) have been extensively investigated in mouse models of autoimmunity. However, the requirements for differentiation and the properties of pathogen-induced human Th17 cells remain poorly defined. Using an approach that combines the *in vitro* priming of naive T cells with the *ex vivo* analysis of memory T cells, we describe here two types of human Th17 cells with distinct effector function and differentiation requirements. *Candida albicans (C.a.)*-specific Th17 cells produced IL-17 and IFN-γ, but no IL-10, while *Staphylococcus aureus (S.a.)*-specific Th17 cells produced IL-17 and could produce IL-10 upon restimulation. IL-6, IL-23, and IL-1β contributed to Th17 differentiation induced by both pathogens, but IL-1β was essential in C.a.-induced Th17 differentiation to counteract the inhibitory activity of IL-12 and to prime IL-17/IFN-γ double producing cells. In addition, IL-1β inhibited IL-10 production in differentiating and in memory Th17 cells, while blockade of IL-1β *in vivo* led to increased IL-10 production by memory Th17 cells. We also show that following restimulation, Th17 cells transiently downregulated IL-17 production through a mechanism that involved IL-2-induced activation of STAT5 and decreased expression of RORγt. Taken together these findings demonstrate that by eliciting different cytokines *C.a.* and *S.a.* prime Th17 cells that produce either IFN-γ or IL-10 and identify IL-1β and IL-2 as pro- and anti-inflammatory regulators of Th17 cells both at priming and in the effector phase (Figure 1).

* ***Zielinski C.E., et al.*** *Nature 2012, 484:514-8.*

This work is done in collaboration with Silvia Monticelli, IRB, and Marco Gattorno, Istituto Gaslini, Genova (IT).

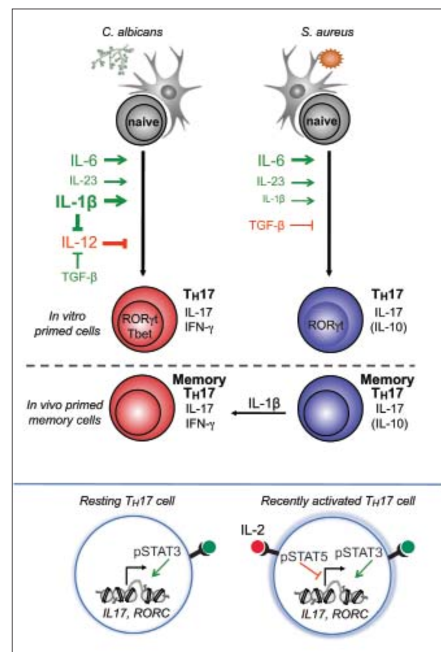

Figure 1
Cytokines regulating differentiation and functional properties of pathogen-specific human Th17 cells.

In vitro and *in vivo* priming of human polarized T cells by microbes

Simone Becattini and Federica Sallusto

The innate immune system is endowed with invariant innate receptors that recognize pathogen-associated or danger-associated molecular patterns (PAMPs and DAMPs, respectively). These receptors are highly expressed in antigen presenting cells (APCs) and regulate the expression of MHC and costimulatory molecules and the production of cytokines that contribute to T cell differentiation, such as IL-1β, IL-6, IL-12, and IL-23. Thus the combination of innate receptors triggered by a given pathogen is thought to determine the class of CD4⁺ T cells elicited, i.e. Th1, Th2, or Th17. While most studies in humans have focused on the effect of single microbial components, much fewer dealt with the effect of whole microbes on APCs, especially with regard to T cell priming. To identify the PAMPs and the antigens associated with a given polarized T cell response we have developed a combined in *vitro/ex vivo* approach to study the mechanisms of microbe-induced T cell priming. Monocytes exposed *in vitro* to microbes prime naïve CD4⁺ T cells, leading to expansion of antigen-specific T cell populations with various effector phenotypes. This heterogeneity, which can be observed even in the same culture, suggests that differentiation cues are delivered unevenly depending on the local milieu. In a parallel approach we isolate memory T cell subsets using chemokine receptors and characterized the T cell response induced by the same pathogens *in vivo*. This approach has led to the identification of a *C. albicans* mannoprotein as an immunodominant antigen recognized by human Th17 cells.

This work is done in collaboration with Laurent Perez, IRB, and Nico Callewaert, Ghent University (BE).

Regulation of cytokine production in human T helper cells

Dominik Aschebrenner, Federico Mele, Christina E. Zielinski, Samuele Notarbartolo and Federica Sallusto

In a previous study, we found that while human resting Th17 clones produced high amounts of IL-17, day 5-activated clones strongly downregulated IL-17 production. At later time points the clones gradually regained the capacity to produce IL-17 as the cells reverted to the resting state. The analysis of transcription factors showed that on day 2 and 5 following restimulation, Th17 clones downregulated *RORC* mRNA expression. In addition, while both resting and day 5 restimulated Th17 clones phosphorylated STAT3 in response to IL-6, only restimulated clones phosphorylated STAT5 in response to IL-2, consistent with the increased expression of CD25. Overexpression of RORγt significantly restored IL-17 production in activated Th17 clones, and restimulation in the presence of a STAT5 inhibitor rescued *RORC* mRNA expression and IL-17 production in a proportion of clones. Collectively, these data suggest that in activated human Th17 cells decreased RORγt expression and increased pSTAT5 - which may compete with pSTAT3 for binding to the *IL17* locus - contribute to the transient downregulation of IL-17 production. Currently, we are extending these studies in two directions. On the one hand we are analyzing Th1 and Th2 clones to understand whether also in these cells cytokine production can be regulated by the activation state of the cell. On the other, we are dissecting the network of signals to find mechanisms of regulation of cytokine gene expression.

This work is done in collaboration with Silvia Monticelli, IRB.

Distribution of pathogen-specific CD4+ T cells within distinct human memory subsets

Federico Mele and Federica Sallusto

We have developed a high throughput cellular screening approach to interrogate the human naïve and memory T cell repertoires against complex antigens, even whole pathogens. The method is based on the polyclonal stimulation and expansion of limiting number of T cells in multiple replicate cultures to generate libraries that can be repeatedly screened for the presence of antigen specific T cells using crude antigens (e.g. allergen extracts or whole pathogens), recombinant protein antigens, or peptides. As APCs we use autologous monocytes, EBV-immortalized B cells, or activated T cells. Using the T cell library method, we are performing a systematic analysis of the frequency, functional properties and distribution of pathogen-specific T cells within different memory subsets. Libraries were established from T cell subsets isolated according to the expression of CD45RO and chemokine receptors and interrogated for reactivity against viral, bacterial, and fungal antigens. We found that i) virus-specific T cells (CMV, HBV, Influenza virus), are present mainly in CXCR3+CCR6+ and CXCR3+CCR6− Th1 subsets; ii) T cells specific for *Staphylococcus aureus and Streptococcus pyogenes* are mainly present in the CXCR3+CCR6+ Th1 and CCR6+CCR4+ Th17 subsets; iii) T cells specific for *Mycobacterium tuberculosis* are almost exclusively present in the CXCR3+CCR6+ Th1 subset; iv) T cells specific for *Candida albicans* are present in the CCR6+CCR4+ Th17 subset and in some donors, are also present in the CCR4+ Th2 subset. A large proportion of C. *albicans*-specific T cells were found to cross-react with the related species *C. dubliniensis* and *C. tropicalis* and some T cells were broadly cross-reactive with even more distant species. Interestingly, *C. albicans*-specific Th2 cells recognize a distinct class of secreted proteins, which are poorly targeted by Th17 cells. Taken together these findings reveal characteristic pathogen-specific as well as and antigen-specific signature within each pathogen.

*** Zielinski C.E., et al.** *Immunol Rev. 2011; 240: 40-51*

This work is done in collaboration with Orlando Petrini, Microbiology Institute, Bellinzona (CH).

Distribution of allergen specific cells in human effector and memory T cell subsets

Tess M. Brodie, Elena Brenna and Federica Sallusto

We are studying the distribution and responsiveness of allergen-specific T cells in different memory subsets in allergic and non-allergic individuals and how the response changes with season. Memory T cell subsets are isolated according to expression of chemokine receptors and libraries are generated and interrogated simultaneously for reactivity against recombinant allergens or peptide pools. Antigen-specific T cell clones are isolated from responding T cell lines and tested for their fine specificity (using recombinant proteins and overlapping peptide libraries) and for their capacity to produce effector cytokines. We expect to gain important information on the distribution, frequency and class of the human T cell response to different allergens and in different stimulatory conditions (natural exposure or vaccination). The information gathered from this analysis is relevant to define with increased precision the quality of the immune response to allergens, and to discriminate the response to natural exposure and specific immunotherapy. This approach may also reveal distinct subsets of Th2 cells characterized by different homing properties.

This work is done in collaboration with Alessandro Sette, La Jolla Institute for Allergy and Immunology, La Jolla, California (US).

Specificity and distribution of self-reactive T cells in health and disease

Daniela Impellizzieri and Federica Sallusto

A long-standing interest in our group is the co-regulation of effector function and migratory capacity in effector and memory T cells. We have originally reported that CCR6 is expressed on Th17 cells, while the skin-homing receptor CCR10 is expressed on T cells producing IL-22 but not IL-17 (operationally defined as Th22). We also reported that in mice, CCR6 is used by Th17 cells to enter the CNS by crossing the epithelium of the choroid plexus, a step that is required to initiate brain inflammation in EAE. Our current aims are: i) To define the specificity and frequency of self-reactive T cells in the naive repertoire of healthy donors; ii) To define the phenotype of self-reactive T cells in patients with autoimmune diseases. We therefore used the T cell library method that was recently developed in our laboratory to study the distribution, frequency and function of autoreactive T cells in patients affected by multiple sclerosis (MS). Blood samples from eight MS patients were obtained and memory T cells were sorted into CCR6+ and CCR6− subsets and libraries of at least 2.5×10^6 T cells/subset were obtained from each of the eight patients. Myelin oligodendrocyte glycoprotein (MOG)-responding T cells were readily detected in most of the patients, albeit at different frequencies, while they were not present in healthy controls. Strikingly, in MS patients, MOG-specific T cells were almost exclusively present in cultures from the CCR6+ T cell subset. T cell clones generated from responding cultures demonstrated that MOG-specific T cells mainly had an IFN-γ+ Th1 phenotype, although some clones were capable of producing IL-17 and GM-CSF. These findings reinforce the notion that pathogenic T cells use CCR6 to gain access in the brain and suggest a therapeutic approach in MS based on the blockade of CCR6.

This project is done in collaborations with Antonio Uccelli, Neuroimmunology Unit, University of Genoa (IT).

Pertussis toxin-driven IL-1β production is required for priming of highly encephalitogenic GM-CSF+ T cells and for EAE pathogenesis

Francesca Ronchi, Camilla Basso, Luana Perlini and Federica Sallusto

IL-1β is a pleiotropic cytokine that plays a role in several inflammatory disorders in humans and in animal models, including mouse experimental autoimmune encephalomyelitis (EAE). It is produced after cleavage of pro-IL-1β by IL-1 converting enzyme (caspase-1), which in turn is activated by a complex of proteins called inflammasome. IL-1β has been shown to be required for differentiation of human and mouse inflammatory Th17 cells characterized by co-expression of IL-17 and IFN-γ. We found that mice deficient for IL-1β or for a component of the inflammasome (the apoptosis-associated speck-like protein containing a caspase recruitment domain, ASC) did not develop EAE following immunization with myelin oligodendrocyte glycoprotein (MOG) in complete Freund's adjuvant (CFA) and pertussis toxin (PT). Autoreactive T cells were primed in wild-type (wt), IL-1β−/− and ASC−/− mice. However, while in wt mice T cells proliferated extensively and acquired the capacity to produce inflammatory cytokines, such as IL-17, IL-22, IFN-γ, and GM-CSF, in IL-1β−/− and ASC−/− mice, cells expanded poorly and showed reduced capacity to produce simultaneously inflammatory cytokines, in particular GM-CSF. Interestingly, induction of polyfunctional (IL-17+ IL-22+ IFNγ+ GM-CSF+) T cells in wt mice was dependent on the presence of PT at the time of immunization. PT was found to rapidly induce IL-1β secretion by CD11c+ and Gr1+ myeloid cells, which are highly recruited in secondary lymphoid organs after *in vivo* PT treatment. Moreover, in mice depleted of Gr1+ myeloid cells, IL-1β produc-

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Fabio Grassi

tion was not induced by PT and priming of polyfunctional T cells was impaired. Taking together, these data support the notion that the disease-inducing effect of PT is due to its ability to induce recruitment of Gr1$^+$ myeloid cells, production of IL-1β, and differentiation of pathogenic polyfunctional T cells.

Cervical lymph nodes and the pivotal role in the CCR6$^+$ T helper cell priming

Camilla Basso and Federica Sallusto

To perform their function, effector and memory T cells have to migrate to sites of antigen challenge. Previous studies have shown that CCR9 is expressed by T cells that migrate to the gut, while CCR10 is expressed on T cells that migrate to the skin. These receptors are induced by dendritic cells (DCs) that process gut derived vitamin A and skin derived vitamin D into active metabolites (retinoic acid and 1,25-dihydroxy-vitamin D3) that elicit CCR9 and CCR10 expression in activated T cells (Figure 2). Using the experimental autoimmune encephalomyelitis (EAE) model, we have recently shown that pathogenic Th17 cells upregulate expression of CCR6 and use this receptor to enter into the CNS by crossing the epithelium of the choroid plexus, which constitutively express the CCR6 ligand CCL20. Based on these findings we hypothesized that CCR6 regulates constitutive migration of lymphocytes in the CNS and that this receptor may be selectively induced in the local microenvironment of the cervical lymph nodes (CLNs). Consistent with this hypothesis, we found that naive CD4$^+$ T cells primed by antigens draining in the CLNs selectively upregulate CCR6 expression. Moreover, *in vitro* experiments showed that CD4$^+$ T cells cultured with cells isolated from CLNs also upregulate CCR6 expression. Migratory DCs seem to be responsible for CCR6 upregulation on T cells, since CCR6 was not induced in CLNs of CCR7 deficient animals which lack this DC subset. We are currently performing experiments to understand what are the signals required for induction of CCR6 expression in T cells primed in the CLNs and to define their cytokine profile.

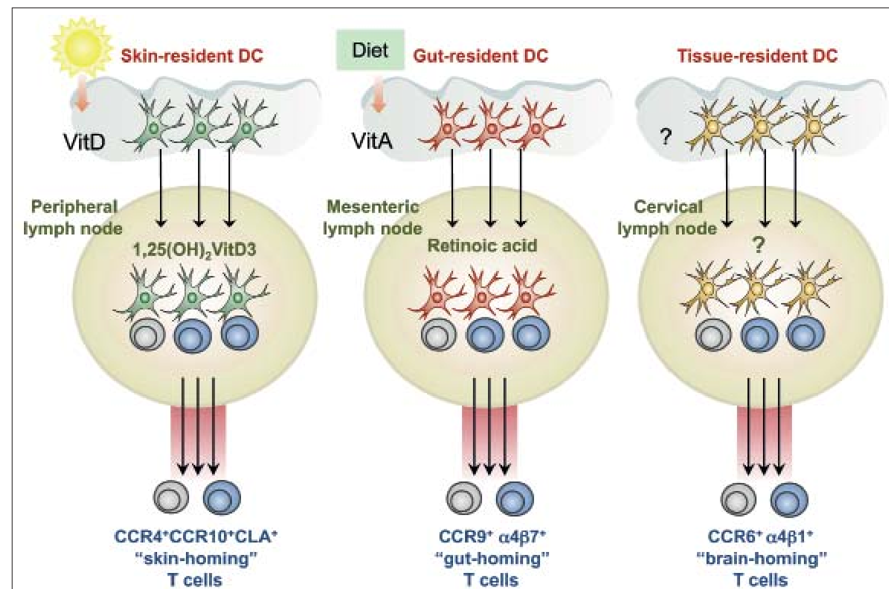

Figure 2. *Imprinting of tissue-specific homing receptors in T cells.*

Overstimulation of T follicular helper leads to impaired antibody response and production of self-reactive antibodies

Dirk Baumjohann, Silvia Preite, Andrea Reboldi, Francesca Ronchi and Federica Sallusto

Cognate T cell-B cell interaction is required for production of high-affinity antibodies and generation of memory B cells and long-lived plasma cells. Here, we investigated T-cell dependent antibody responses in lymphopenic CD3ε$^{-/-}$ mice adoptively transferred with naïve CD4$^+$ T cells. Upon immunization, OT-II cells preferentially differentiated into follicular helper T (Tfh) cells, leading to strong and sustained germinal center (GC) reactions. Using different experimental approaches we show that maintenance of the Tfh phenotype was dependent on sustained antigenic stimulation and required GC B cells. Although Tfh cells induced isotype switch and short-lived plasma blasts, they failed to induce affinity-maturation and long-lived plasma cells. Rather, Tfh cells provided help in a bystander fashion leading to hypergammaglobulinemia, hyper IgE, and production of poly- and self-reactive antibodies. These data provide mechanistic insights into Tfh generation and maintenance and a link between overstimulation of Tfh cells and development of dysregulated humoral immune responses.

This work is done in collaboration with Antonio Lanzavecchia, IRB.

CXCR5$^+$ follicular helper T cells: generation, trafficking and relationship with other T cell lineages

Gabor Gyülvészi, Tomasz Wypych and Federica Sallusto

Chevalier N. et al. * *J Immunol. 2011; 186: 5556-68.*

The stability of CXCR5 expression in the primary response and in the memory phase is an important determinant of immune homeostasis and is of immediate relevance to the question of whether Tfh cells represent a distinct, stable cell lineage or a transient activation state. Genetic fate mapping provides means to evaluate both lineage stability and information on mechanisms of tissue maintenance *in vivo*. We are using this approach to evaluate Tfh lineage stability in primary and memory phases of the immune response by generating knock-in mice harbouring a cassette containing an internal ribosome entry site (IRES) followed by DNA sequence encoding a “triple” fusion protein, formed by enhanced green fluorescent protein (eGFP), Cre recombinase and mutated human estrogen receptor ligand-binding domain (ERT2), inserted into the 3′ untranslated region (UTR) of the CXCR5 gene. The Cxcr5eGFP-Cre-ERT2 mice will be bred to mice that express a Cre recombination reporter allele of the ubiquitously expressed ROSA26 locus containing a loxP site–flanked stop-cassette followed by a DNA sequence encoding yellow fluorescent protein (YFP). In these mice, the GFPCreERT2 fusion protein will be sequestered in the cytosol, and therefore YFP will not be expressed. Treatment with tamoxifen will allow for nuclear translocation of the fusion protein, excision of the floxed stop-cassette, and constitutive and heritable expression of YFP in a population of cells that express CXCR5 at the time of tamoxifen administration. The generation of the reporter mouse can be achieved through the traditional homologous recombination in ES cells or through the use of an innovative method, the TALEN nucleases. We decided to apply the second approach which enables us to omit the ES cell work and to directly inject the TALENs together with the targeting construct into oocytes. The advantage of this method is the increased recombination frequency and shorter time frame. We have already generated the targeting vector which contains the reporter sequence (IRES-eGFP-CreErt2) and the two short homology arms which are required for proper integration. The reporter sequence will be incorporated into the 3’ UTR

of the *cxcr5* gene after the stop codon directly. We decided to keep the original 3' UTR polyadenylation signal in order not to interfere with the native integrity of our gene of interest. Currently the TALEN nuclease is being generated and will be microinjected together with the plasmid.

This work is done in the context of the ProDoc project and is done in collaboration with Jens Stein and Britta Engelhardt, TKI, Bern (CH).

Chemokine receptors on innate lymphoid cells for control of tumor growth

Gabor Gyülvészi and Federica Sallusto

It is known that ectopic expression of IL-12 protects the host against different types of tumors. This effect seems to be mediated by a recently characterized subset of lymphoid tissue–inducer (LTi) cells expressing the natural cytotoxicity receptor (NCR) NKp46 and producing IL-22. Lymphoid tissue–inducer cells express several chemokine receptors which are important during their life time. CCR6 and CXCR5 are the most prominent and well characterized receptors on the surface of these cells. CCR6 plays an essential role to direct LTi cells to Peyers patches where they form part of the intestinal immune system. In contrast, CXCR5 takes part in the formation of lymphoid structures during development by driving LTi cells towards CXCL13 expressing stroma. Nothing is known on which chemokine receptors are required to mediate the anti-tumor effect of LTi cells. We are addressing this question using the B16 mouse melanoma model. Equal number of B16 or B16-IL-12 melanoma cells were injected into $CCR6^{-/-}$, $CXCR5^{-/-}$ and wt hosts and tumor growth was monitored. In $CCR6^{-/-}$ mice there was no difference in tumor growth compared to wt mice while, unexpectedly, in $CXCR5^{-/-}$ mice both parental B16 and B16-IL12 tumor growth were significantly increased. Analysis of tumor infiltrating lymphocytes, however, did not reveal difference between wt and $CXCR5^{-/-}$ mice; LTi cells were present in both mice and in comparable number. Activation of CXCR5 by its ligand CXCL13 has been shown to be required to activate VLA-4 and promote adhesion to its receptor VCAM-1 on tumor cells. This notion led us to hypothesize that CXCR5 may function by promoting VLA-4-VCAM adhesive interaction. However, VLA-4 blocking antibody (PS/2) did not have any effect during tumor development. Currently we are isolating tumor infiltrating lymphocytes, and analyzing their chemokine expression pattern. We hope to find a candidate receptor which is selectively up regulated on lymphocytes in the IL-12 expressing tumor. This screening might enable us to depict the pathway which initiates the efflux of LTis into the tumor mass.

This work is done in collaboration with Burkhard Becher, University of Zurich (CH).

Funding

Bill and Melinda Gates Foundation
PTVDC: Pox T Cell Vaccine Discovery Consortium / 2006-2011

Institute of Arthritis Research
The role of T and B cells in arthritis / 2009-2011

European Union
NEWTBVAC: Discovery and Pre-Clinical Development of New Generation Tuberculosis Vaccines
241745 / 2010-201

European Union
IDAMS: International Research Consortium on Dengue Risk Assessment,
Management, and Surveillance
281803 / 2011-2016

Swiss National Science Foundation
Effector and memory T cell subsets in man and mouse
3100A0-131092 / 2010-2013

Swiss National Science Foundation
ProDoc Cell Migration
PDFMP3-137127 / 2011-2014

Collaborations

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Visiting scientists

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Publications

Human antibodies against dengue enhance dengue viral infectivity without suppressing type I interferon secretion in primary human monocytes.
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Zielinski C.E., F. Mele, D. Aschenbrenner, D. Jarrossay, F. Ronchi, M. Gattorno, S. Monticelli, A. Lanzavecchia, and F. Sallusto.
Nature. 2012; 484:514-8.

Lectures and Seminars

Keystone Symposium "Tuberculosis: immunology, cell biology and novel vaccination strategies"
Vancouver (Canada) / 15-20.01.2011

The University of Birmingham Alumni Lecture in Immunology
Lecture: T cell differentiation, migration, and immune regulation"
Birmingham (UK) / 27.01.2011

Keystone Symposium "Immunologic memory, persisting microbes and chronic disease"
Banff (Canada), February 06-11.02.2011

Keystone Symposium "Dendritic cells and the initiation of adaptive immunity"
Santa Fe, NM (US) / 12-17.02.2011

Imperial College London
Centre for Respiratory Infections (CRI) Distinguished Seminar Series: "Dissecting the human T cell response to microbes"
London (UK) / 08.03.2011

15th Annual Woods Hole Immuno Parasitology Conference
Keynote Lecture
Woods Hole, MA (US) 17-20.04.2011

6th ENII Summer School in Advanced Immunology, "Homeostasis and Perturbations of Immunity"
Capo Caccia, Alghero (IT) / 15-22.05.2011

Institut Pasteur
Seminar "Dissecting the human T cell response to microbes"
Paris (FR) / 16.05.2011

Oxford University, The Weatherall Institute of Molecular Medicine
MRC HIU Seminar Series "Novel approaches to analyse the human T cell response to microbes"
Oxford (UK) / 23.05.2011

University of Erlangen Medical School
Immunological Seminar Series: "Dendritic cells and the demanding task of priming T cell responses"
Erlangen (DE) / 31.05.2011

30th EAACI Congress
Istanbul (TK) / 11-15.06.2011

FOCIS 2011
Washington, DC (US) / 23-26.06.2011

3rd Hellenic Immunology Summer School
Spetses Island (GR) / 27.06-1.07.2011

EMBO Course "Signaling in the Immune System"
Siena (IT) / 10-14.09.2011

Harvard Medical School, Department of Microbiology and Immunobiology
Seminar "Dissecting human T cell response in immunity and autoimmunity"
Boston (US) / 18.10.2011

Annual Meeting of the French Society for Immunology
Montpellier (FR) / 8-10.11.2011

Annual Meeting of Japanese Society for Immunology
Chiba (JP) / 27-29.11.2011

6th International Congress "Psoriasis: from Genes to Clinic"
London (UK) / 1-3.12.2011
Annual Congress of the British Society for Immunology
Liverpool (UK) / 5-9.12.2011

14th International Conference on Lymphocyte Activation and Immune Regulation "T cell differentiation and plasticity"
Newport Beach, CA (US) / 3-5.02.2012

Keystone Symposium, "Th17 Cells in Health and Disease"
Keystone, CO (US) / 5-10.02.2012

University of California San Francisco (UCSF) Immunology Seminar Series "T lymphocyte differentiation, migration, and immune regulation"
San Francisco, CA (US) / 13.02.2012

Marcus Thelen
Signal Transduction

Marcus Thelen

Marcus Thelen studied biochemistry at the University of Tübingen (DE). He received his PhD from the University of Bern. He then moved to the Theodor-Kocher-Institute in Bern where his interests focused on inflammation and chemokines. In 1989, he went to the Rockefeller University in New York, joining the group of Alan Aderem in the Laboratory of Cellular Physiology and Immunology of Cohn/Steinman department. Biochemical aspects of cyotokine- and endotoxin-mediated phagocyte priming and cytoskeleton-mediated signal transduction were the topics of his studies. In 1992, he received a career development award (START) from the Swiss National Science Foundation and returned to the Theodor-Kocher-Institute at the University of Bern. He created his own research group working on molecular mechanisms of signal transduction in leukocytes, focusing on PI 3-kinase-dependent pathways and chemokine-mediated receptor activation. He obtained the *venia docendi* in 1994 and was awarded an honorary professorship in 2001 from the University of Bern. 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 fundamental role in regulating leukocyte trafficking. Inflammatory chemokines orchestrate the recruitment of immune cells to sites of inflammation while their homeostatic counterparts mediate leukocytes homing to lymphoid organs and bone marrow. Typically cell migration is governed by chemotactic cues consisting of chemokines which are produced by various cell types, such as endothelium, epithelium and stromal cells, and are often retained at their surface. While most chemokine receptors follow a common paradigm of cell activation, more recently a small group of atypical chemokine receptors was described. Their function is to scavenge chemokines and therefore balance the activity of chemokines. For the proper resolution of an immune response, scavenging of chemokines is important to cease the recruitment of inflammatory cells and to dampen the response. During development, the atypical receptors can carve gradients and restrict the availability of chemokines for their cognate receptors, thereby controlling cell positioning. The laboratory investigated the function and molecular mechanisms of classical and atypical chemokine receptors in mediating leukocyte migration in health and disease.

Team

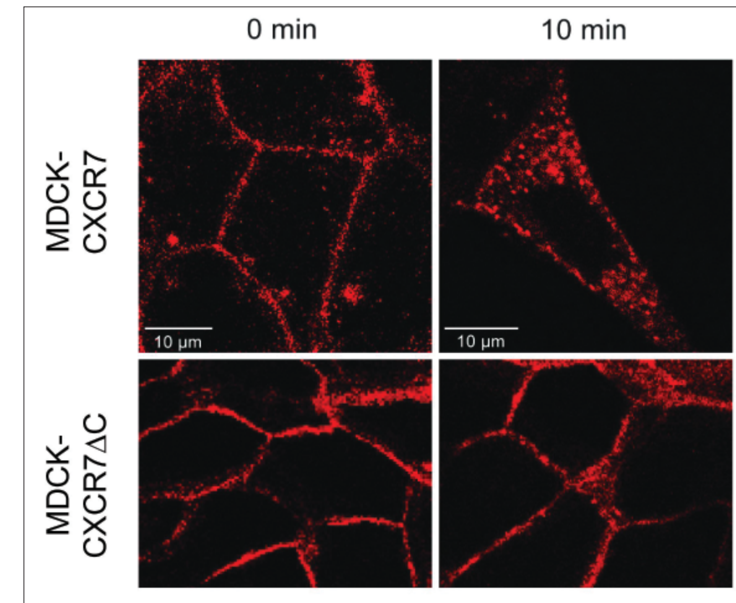
Group Leader: Marcus Thelen, PhD > marcus.thelen@irb.usi.ch
Members: Marie-Luise Humpert, PhD student - Sylvia Thelen, PhD

Molecular mechanisms of CXCR7 sorting and potential signaling properties.

Marie-Luise Humpert and Marcus Thelen

Chemokines receptors couple to pertussis toxin sensitive Gi proteins to trigger cellular responses.

Within the chemokine system CXCL12 and its signaling receptor CXCR4 possess exceptional properties. Genetic deletion of either molecule leads to a similar lethal phenotype, which is exceptional as deletion of no other receptor or chemokine is fatal within the chemokine system. The phenotype is characterized by markedly impaired lymphopoiesis and myelopoiesis, imperfect vasculature, abnormal brain and heart development leading to perinatal death. These findings led to the assumption that CXCR4 and CXCL12 represent a monogamous receptor-chemokine pair. In addition expression of CXCR4 strongly correlates with the metastatic potential of diverse tumor cells. Among chemokine receptors, CXCR4 has unique signaling properties capable of promoting the sustained activation of intracellular signaling cascades, which is strictly dependent on the availability of extracellular CXCL12.
Atypical chemokine receptors share the heptahelical structure of rhodopsin-like receptors, but do not couple to G-proteins. Through their decoy function these receptors eliminate chemokines from the environment preventing inflammation or contributing to the resolution of inflammation. Accordingly, it was proposed that the function of this class of receptors is to regulate innate and adaptive immune responses by balancing the availability of chemokines for leukocyte trafficking. CXCR7, the unique decoy receptor for the homeostatic chemokine CXCL12, is expressed on leukocytes and endothelium. The receptor was shown to affect CXCR4 function by regulating the availability of the ligand and might also directly interact with CXCR4.
Our laboratory recently described CXCR7 as scavenger for the chemokines CXCL12 and CXCL11. Despite the lack of signaling through G-proteins, CXCR7 may use biased signaling though arrestins. The receptor plays a critical role in development, as targeted deletion in mice is lethal. The failure of coupling to G-proteins is consistent with the assumption that CXCR7 acts primarily as a scavenger. We provided formal evidence for such activity in mammalian cells. The current project focuses on investigations analyzing the mechanism of CXCR7-dependent chemokine scavenging and its role in a physiological context.
The project focuses on the following topics:
- *Function of CXCR7 in leukocytes*. We recently confirmed the expressions and function of CXCR7 on primary human B cells. Ongoing studies investigate the subpopulation of human and mouse B cells with highest expression and functional activity *in vitro* and *in vivo*. Studies will reveal a regulatory activity of CXCR7 and CXCR4-mediated leukocyte migrations.
- *Investigations of ligand-dependent and -independent receptor trafficking to elucidate the mechanism of chemokine scavenging*. Intracellular trafficking of CXCR7 is remarkably different from CXCR4. The temporal and molecular mechanisms of receptor sorting are not well characterized. With the aid of receptors fluorescently tagged at their N-terminus, fluorescent ligands, and fluorescent tagged markers of endosomal compartments the steps of cargo sorting will be analyzed. Current investigations assign the C-terminus a critical role in receptor trafficking.
- *Identification of potential CXCR7-mediated atypical signal transduction leading to intracellular protein phosphorylation*. Based on the notion that CXCR7-cycling is ligand enhanced and that phosphorylation events are associated with almost any receptor-mediated cell activation, ligand-induced phosphorylation events are expected, which shall allow tracking of targeted proteins and eventually the involved pathways.



Transfected MDCK cells (MDCK-CXCR7, upper and MDCK-CXCR7ΔC, lower) were labelled with sfp synthase (PPTasa) and CoA-Atto647N for 20 min at 17°C and then shifted to 37°C for 0 min (left) and 10 min (right). Cells were fixed with paraformaldehyde for microscopy.

The guanine nucleotide exchange factor for Rac P-Rex1

Sylvia Thelen and Marcus Thelen

The small GTPases of the Rho family, Rac and Cdc42, are critical for rapid rearrangements of the actin cytoskeleton observed during filopodia and lamellipodia formation in migrating cells. The GTPases act as switches and are either 'on' in their GTP bound form or 'off' when loaded with GDP. Activation of the GTPases is catalyzed by specific GTP exchange factors (GEF). The phosphatidylinositol 3,4,5-trisphosphate (PIP_3)-dependent exchanger 1 (P-Rex1) is assumed to be involved in G-protein coupled receptor (GPCR)-mediated Rac activation. P-Rex1 activity is stimulated by the PI 3-kinase product PIP3 and by the βγ subunits of heterotrimeric G-proteins, which are released upon activation of GPCRs. Consistent with the activation by these cofactors and their cellular localization following stimulation of the cells, P-Rex1 is recruited to the plasma membrane. Overexpression of P-Rex1 or its suppression by siRNA markedly alters chemokine-stimulated migratory capacity of myeloid leukocytes, consistent with the assumption that 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.
Investigations shall reveal the function of different P-Rex1 domains and their role in chemokine receptor-mediated signal transduction. Mouse bone-marrow derived hematopoietic precursor cells from animals lacking either the expression of P-Rex1 and/or P-rex2 are arrested by the conditional expression of HoxB8 and HoxA9. Various P-Rex1 mutants are transduced into these cells and differentiated later into neutrophils and monocytes. Assessment of the chemotactic responsiveness allows to delineate the function of different P-Rex domains.

G-protein coupled receptor activity in migrating cells

Marcus Thelen and Sylvia Thelen

Cell migration is a well-described phenomenon that is critical during development, tissue repair, and immune homeostasis, surveillance and responses. In general, cells migrate along a guidance cue, which is formed by a chemotactic gradient. The relative concentration difference of a chemoattractant between the front and the rear of a motile cell within such gradient is minute. Nevertheless, cells can easily sense and polarize along the gradient forming a characteristic leading edge and a trailing uropod. Intracellular polarization is realized by locally activated enzymes, *e.g.* PI 3-kinase activity is stimulated in the front whereas pathways dependent on the small GTPase Rho are activated at the rear. Leukocytes rapidly assume the typical morphology when moving in a shallow gradient of chemoattractants. The small concentration difference of the chemoattractant along the polarization axis is probably insufficient to stimulate different receptor efficiencies at the front and the rear. To explain the functional morphology, several mechanisms for cell polarization have been proposed: an asymmetric receptor distribution, local differences in receptor occupancy or intracellular feedback signaling mechanisms. Most chemotactic movement in mammalian cells is stimulated via Gi-protein-coupled receptors (GPCRs). Confocal section analyses reveal an equal distribution of chemoattractant receptors over the entire plasma membrane in cells moving along a chemotactic gradient. Receptor activation is typically followed by its desensitization and internalization. In migrating human monocytes, receptors at the leading edge remain responsive allowing the continuous migration in a gradient. Nevertheless, during chemotaxis, monocytes actively internalize chemokines by a receptor-dependent mechanism. In order to retain responsiveness of the cells to the chemotactic gradient, the receptors are not desensitized, but rather must cycle back to the cell surface after delivery of the cargo to lysosomes. Uptake of chemoattractant is independent of PI 3-kinase activity and does not require coupling of the receptors to the G-protein. The observations suggest a scavenging mechanism of chemotactic receptors during migration leading to the consumption of the chemotactic gradient.

Funding

Ticino Foundation for Cancer Research
Detailed study of the interactions and subcellular distribution of the tumorigenic chemokine receptor CXCR7/RDC1 in lymphocytes
2009-2011

Gottfried und Julia Bangerter-Rhyner-Stiftung
Molecular mechanisms of CXCR7 sorting and potential signaling properties
Jost Reinhold Foundation
2010-2013

Novartis Foundation for Medical Biological Research
Plasmablast Differentations: role of CXCR7
2012

Collaborations

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Publications

Cxcl12 evolution - subfunctionalization of a ligand through altered interaction with the chemokine receptor.
Boldajipour, B., M. Doitsidou, K. Tarbashevich, C. Laguri, S.R. Yu, J. Ries, K. Dumstrei, S. Thelen, J. Dorries, E.M. Messerschmidt, M. Thelen, P. Schwille, M. Brand, H. Lortat-Jacob and E. Raz.
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Proteomics. 2012; Epub May 23, 2012

CCR2 Acts as Scavenger for CCL2 during Monocyte Chemotaxis.
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PLoS One. 2012; 7:e37208.

Lectures and Seminars

University of Lausanne, CHUV
Seminar “Functional chemokine receptor responses”
Lausanne (CH) / 18. 03. 2011

Annual Meeting of the German Society for Pharmacology and Toxicology (DPTG)
Frankfurt / 31.03.2011

Centro de Biología Molecular Severo Ochoa (CSIC - University of Madrid)
Seminar "The Yin Yang of Chemokine Receptor Functions"
Madrid (ES) / 28.04.2011

Paul-Scherrer-Institute
Seminar "The Yin Yang of Chemokine Receptor Functions"
Villigen (CH) / 11.05.2011

University of Konstanz
Seminar "The Yin Yang of Chemokine Receptor Functions"
Konstanz (DE) / 26.05.2011

University of Jena
Seminar "CXCR7: expression and function"
Jena (DE) / 07.11.2011

STS-Meeting Weimar
Keynote lecture
Weimar (DE) / 09.11.2011

Mariagrazia Uguccioni

Chemokines in Immunity

Mariagrazia Uguccioni

Mariagrazia Uguccioni received a degree in Medicine from the University of Bologna (IT) where she specialized in Haematology in 1994. From 1993 to 2000 she was a member of the Theodor Kocher Institute, University of Bern (CH), and since 2000 she is group leader at the IRB. She is member of the Bologna Academy of Science since 2009. 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 inflammatory diseases, tumours, 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 an emphasis on the mechanisms governing fine-tuning modulation of their expression and activity. Chemokines are secreted proteins and have emerged as key controllers of integrin function and cell locomotion. The effects of chemokines are mediated by seven transmembrane domain receptors coupled to GTP-binding proteins, which are differentially expressed in a wide range of cell types. The resulting combinatorial diversity in responsiveness to chemokines guarantees the proper tissue distribution of distinct leukocyte subsets under normal and inflammatory/pathological conditions. A vast range of *in situ* experiments, aimed at understanding which chemokines are produced in specific circumstances, has revealed that a variety of chemokines can be concomitantly produced at target sites of leukocyte trafficking and homing. This renders the chemokine system a good target for therapy, and has increased the search also by pharmaceutical companies for small molecule chemokine antagonists. While we understand the effects of different chemokines individually, much less is known about the potential consequences of the expression of multiple chemokines, cytokines, toll-like receptor ligands or other inflammatory molecules on leukocyte migration and function. Our group discovered the existence of additional features of chemokines: their ability to antagonize or enhance, as synergy-inducing chemokines, the activity of other chemokines. Recently we have discover that the alarmin HMGB1 can enhance chemokine activities and be essential in the first phase of cell influx in injured tissues.

Team

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Members: Maria Gabriela Danelon, Technician - Milena Schiraldi, PhD student - Denise Bottinelli, PhD student - Valentina Cecchinato, PhD - Camilla Marini, MSc

Chemokines: Structure/Function Studies

Milena Schiraldi, Camilla Marini, Gabriela Danelon and Mariagrazia Uguccioni

Chemokine structure/function studies led us to identify chemokines that can act as natural antagonists by preventing natural agonist binding and the subsequent activation of the receptor. Recently, we have described chemokines that can act in synergism with chemokine receptor agonists, forming heterocomplexes able to induce functional responses at lower agonist concentration. Several mechanisms have been proposed by us and other groups to provide an explanation for the synergy between chemokines: Dual receptor-mediated chemokine synergy and chemokine heterocomplexes (Figure 1).

After tissue damage, inflammatory cells infiltrate the tissue and release pro-inflammatory cytokines. HMGB1, a nuclear protein released by necrotic and severely stressed cells, promotes cytokine release via its interaction with the TLR4 receptor, and cell migration via an unknown mechanism. We show that HMGB1-induced recruitment of inflammatory cells depends on CXCL12. HMGB1 and CXCL12 form a heterocomplex, which we characterized by nuclear magnetic resonance and surface plasmon resonance, that acts exclusively through CXCR4 and not through other HMGB1 receptors. FRET data show that the HMGB1/CXCL12 heterocomplex promotes different conformational rearrangements of CXCR4 from that of CXCL12 alone. Mononuclear cell recruitment *in vivo* into airpouches and injured muscles depends on the heterocomplex and is inhibited by AMD3100 and glycyrrhizin. Thus, inflammatory cell recruitment and activation both depend on HMGB1 via different mechanisms.

* *Schiraldi M. et al.* J Exp Med. 2012, 209: 551-63. -Highlights, Nat. Rev. Immunol. 2012, 12: 232.

* *Gouwy M. et al.* Immunol Lett. 2012, in press.

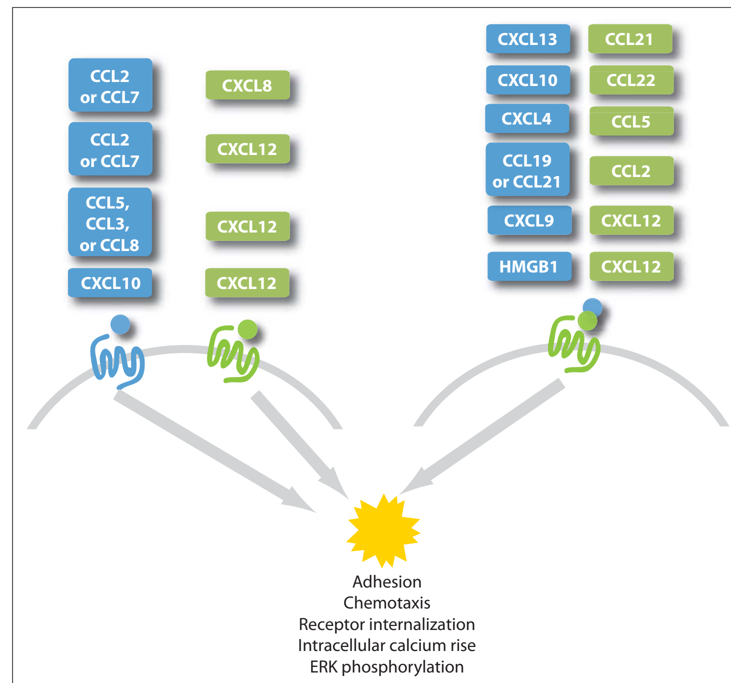

Figure 1
Model of action in human chemokine synergism. Two different models have been proposed to provide an explanation for the synergism between chemokines leading to different human leukocyte responses.

Chemokines in HIV/SIV infection and vaccination strategies

Valentina Cecchinato, Denise Bottinelli, Gabriela Danelon, Federica Sallusto and Mariagrazia Uguccioni

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 the 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 the natural host and in pathogenic models of SIV infection. A decrease in the frequency of Th17 cells, a recently discovered subset of effector T cells involved in the immune response against extracellular bacteria, has been described by Dr. Cecchinato 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. In the frame of a project funded by the European Community, we are investigating the mechanisms that mediate Th17 cells 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 and in view of generating efficient vaccines.

Moreover, *in vivo* studies are performed in collaboration with the group of Dr. Sallusto to characterize, in the draining lymphoid organs at early phase, the *in situ* expression of cytokines and chemokines upon subcutaneous injection of adjuvants, which could account for the different cellular responses and identify molecules to be used as markers for the different adjuvant activities.

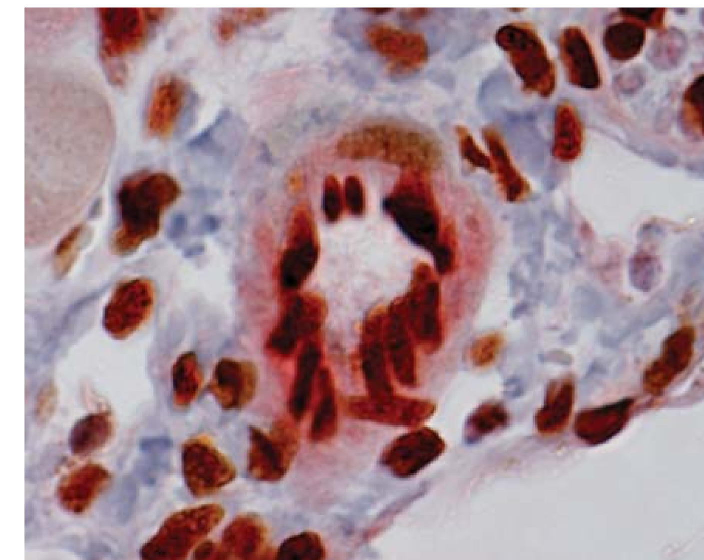

Figure 2
CXCL12 (red) and HMGB1 (brown) protein expression in a vessel from a cardiotoxin injured muscle. Double immunohistochemistry performed on paraffin-embedded tissues. Magnification 40x.

Funding

European Union
ADITEC: Advanced Immunization Technologies
FP7 - 280873 / 2011-2015

European Union
Marie Curie IEF Fellowship to Valentina Cecchinato
FP7 - PEOPLE-IEF-2008 / 2009-2012

Swiss National Science Foundation
Impact of multiple chemokine expression in human disease
3100A0- 118048-1 / 2008-2011

San Salvatore Foundation
The role of chemokine synergy-inducing molecules in controlling the tumour microenvironment , cell migration and metastasis
2011-2014

Collaborations

Marco Bianchi
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Mario Mellado
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Costantino Pitzalis
William Harvey Institute, London (UK)

Visiting Scientists

Antonella Antonelli
San Raffaele Scientific Institute, Milan (IT)

Barbara Celona
San Raffaele Scientific Institute, Milan (IT)

Publications

HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4.
Schiraldi M., A. Raucci, L. Martínez Muñoz, E. Livoti, B. Celona, E. Venereau, T. Apuzzo, F. De Marchis, M. Pedotti, A. Bachi, M. Thelen, L. Varani, M. Mellado, A. Proudfoot, M. Emilio Bianchi and M. Uguccioni.
J Exp Med. 2012; 209: 551-63.

Role of lymphoid chemokines in the development of functional ectopic lymphoid structures in rheumatic autoimmune diseases.
Corsiero E., M. Bombardieri, A. Manzo, S. Bugatti, M. Uguccioni, C. Pitzalis. (Review).
Immunology Letters. 2012; in press.

Possible mechanisms involved in chemokine synergy fine tuning the inflammatory response.
Gouwy M., M. Schiraldi, S. Stuyf, J. Van Damme and M. Uguccioni. (Review).
Immunology Letters. 2012; in press.

Lectures and Seminars

BIC seminars
Seminar "Chemokines in skin diseases"
Bern (CH) / 27.04.2011

Istituto di Ricerca Codivilla-Putti
Seminar "Synergy-inducing chemokines: a new model for chemokine interactions"
Bologna (IT) / 29.06.2011

EADV Meeting
Bellinzona (CH) / 11.11.2011

Luca Varani
Structural Biology

Luca Varani

Luca Varani graduated in chemistry at the University of Milan (Italy) with a thesis in structural biology. He then moved to the MRC-Laboratory of Molecular Biology and obtained a PhD degree at the University of Cambridge (UK) in 2000. His PhD research focused on the role of RNA and protein interactions in the regulation of gene expression at the post-transcriptional level, culminating in the determination of the largest NMR structure and one of only three 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 (USA) as a postdoctoral fellow and was awarded an "EMBO Fellow" 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 Institute for Research in Biomedicine (Bellinzona, CH) as a group leader in Structural Biology.

Research Focus

Our group uses computational, biochemical and biophysical tools to determine the structure of proteins and characterize their interactions with other molecules, with particular attention to antibody-antigen interactions in infectious diseases.
Experimental techniques like nuclear magnetic resonance (NMR) and X-Ray crystallography have been traditionally used to investigate biomolecular structures at the atomic level. 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 is constantly increasing.
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 to merge biochemical data, experimental structural validation and computational docking in an efficient workflow, and to apply it to biologically relevant cases such as the interactions between antibodies and pathogens or between chemokines, proteins responsible for controlling cellular trafficking.

Team

Group Leader: Luca Varani, PhD > luca.varani@irb.usi.ch
Members: Elsa Livoti, PhD student - Mattia Pedotti, PhD - Luca Simonelli, PhD - Zinaida Yudina, PhD student - Daniela Iannotta, PhD student - Marco Bardelli, PhD student

Prediction and characterization of antibody-protein interactions in Dengue Virus

Luca Simonelli, Mattia Pedotti, Elsa Livoti and Luca Varani

Individuals that survive a viral infection have antibodies (Abs) capable of detecting and neutralizing subsequent attacks by the same virus. These Abs bind antigens (Ags), often viral proteins, through specific atomic interactions between the Ab and the region of the Ag that it recognizes (i.e. epitope). A better understanding of these interactions is expected to accelerate vaccine development, since most current vaccines are based on the generation of neutralizing Ab responses.
If we understand the structural rules governing Ab-Ag interactions to a given virus, then we have the molecular basis to attempt to design and synthesize new epitopes to be used as vaccines or optimize the antibodies themselves for passive immunization. Comparing the binding of several different antibodies to related Ags should also further our understanding of general principles of recognition.
We recently proposed an experimentally validated computational approach for the rapid and systematic characterization of Ab-Ag complexes (1). Schematically, we isolate Abs from the blood of human donors infected with a given virus; produce and purify milligramme quantities of human monoclonal antibodies (in collaboration with A. Lanzavecchia); characterize their immunological and biophysical properties; determine their epitope through NMR epitope mapping and use the NMR results to drive and validate computational docking simulations of their complex with the desired antigen. Finally, the structural analysis of the complexes is the starting point for the design of antibody mutations aimed at modifying their properties in a predictable manner, with the goal of validating our results and engineer new antibodies with improved properties (Figure 1).

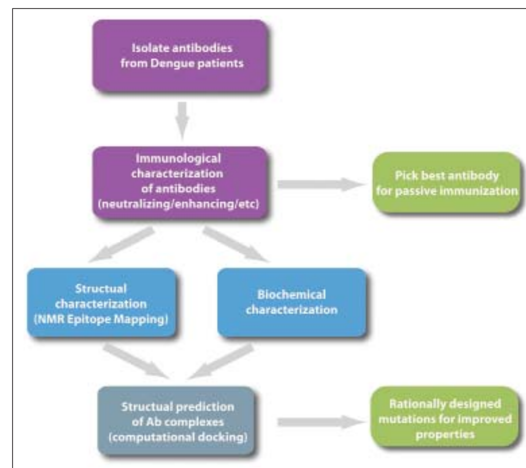

Figure 1
Experimental workflow

Dengue Virus: a case study

Dengue Virus (DENV) is a flavivirus responsible for 100 million annual human cases, including 500,000 hospitalizations and 20,000 deaths with an economic burden rivaling that of malaria. Although DENV has been mainly restricted to the tropical region, both its epidemic activity and its geographic expansion are increasing as travel, urbanization and climate changes create favorable conditions for vector and virus dissemination. An estimated 2.5 billion people are at risk of infection.

No cure or vaccine for DENV is currently available. The effort to find one has been hampered by the presence of four different dengue serotypes (DENV1–4) and by a poorly understood process almost unique in human medicine: antibody-dependent enhancement (ADE). Abs raised against a previous Dengue infection facilitate subsequent infection by a different serotype and lead to dengue hemorrhagic fever, an often lethal form of the disease. This feature complicates the task of finding a vaccine, since a vaccine that would not protect equally against all four serotypes would actually contribute to the emergence of dengue hemorrhagic fever. At the structural level, the most interesting region to study is the so-called Domain III of Dengue E protein (DIII), which forms the surface of the virus. DIII is the main target of neutralizing antibodies against DENV and it is relatively small, making it ideal for NMR and computational studies.
Our aim is to compare a large number of antibodies bound to DIII of the four Dengue serotypes, searching for correlations between immunological and structural trends and exploiting them to further our understanding of antibody-antigen interaction and ADE, as well as a basis for drug design and improved vaccine strategies. In a simplistic example, should we find that all Abs effective against DENV4 have a positive charge in a particular three-dimensional position, we would try to introduce such a charge in Abs lacking it, thus improving their characteristics. Conversely, should all effective Abs against a certain serotype recognize a particular epitope, then it is conceivable to prepare an antigen sharing the best epitopes of each serotype as a possible vaccination agent.
This work is done in collaboration with Antonio Lanzavecchia and Federica Sallusto, IRB.

One antibody, four serotypes, two binding modes

The monoclonal antibody DV21.5 was isolated from a human donor that recovered from Dengue virus. The antibody binds to all four existing Dengue serotypes and we determined its binding region on each serotype with NMR epitope mapping. We then used this information to drive and validate computational docking predictions of the complexes between DV21.5 and four different antigens: domain III of the surface protein from each Dengue serotype. The antibody uses two different binding modes to interact with Dengue and this correlates to its immunological properties: it has a similar interaction with serotype 1 and 3, which it can neutralize, whereas it binds differently to serotype 2 and 4, which are not effectively neutralized. Analysis of our computational models allowed us to design single point mutations in the antibody in order to modify its properties in a predictable manner. We were able to selectively inhibit its binding to a given serotype and, most importantly, to design a mutated antibody with a 10-fold increase in virus neutralization potential. The work proves that experimentally validated computational docking is an accurate, rapid and powerful tool for the characterization and rational engineering of antibodies (Figure 2).

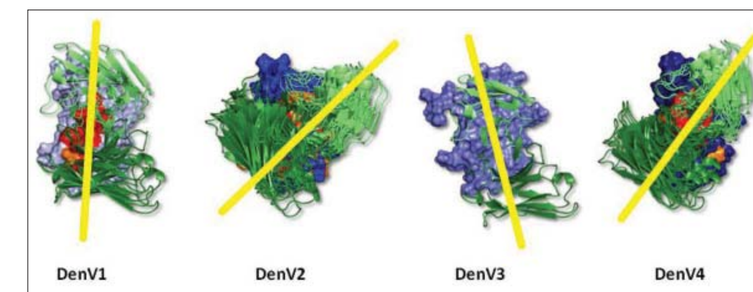

Figure 2
Antibody DV21.5 binds to the four existing Dengue serotypes with different orientations. Top: the antibody is shown in green over the surface of domain III from each serotype (blue). Yellow bars help to identify the general binding orientation. All the computational models that agree with the experimental data are shown as an overlay for each serotype.

Characterization of antibody-protein interactions in Diphtheria Toxin

Zinaida Yudina, Mattia Pedotti and Luca Varani

Diphtheria is an acute infectious disease caused by the bacterial Diphtheria Toxin (DT). Although mass immunization has virtually eradicated diphtheria from the western world, the disease continues to be a serious health threat in regions like the former USSR, Asia and South America. In the 1990s, for instance, an epidemic caused approximately four thousand deaths in Russia even amongst formerly vaccinated individual, apparently due to a decline in adult immunity level. Beside the medical implications, the diphtheria toxin has been extensively characterized at the biochemical level and represents a good model for the study of antibody-toxin interactions. Curiously, however, there is no structural information on DT-antibody complexes. The Lanzavecchia group has isolated a number of human monoclonal antibodies with a remarkably strong binding affinity for DT. Some of these antibodies are very potent neutralizers but, intriguingly, they are not those with the stronger binding affinity according to preliminary surface plasmon resonance (SPR) measurements. Here we aim to use experimentally validated computational docking to characterize the interaction of these antibodies with DT at the structural level. Since the convenience of NMR epitope mapping (see earlier description) is limited by the large size of DT, peptide mapping and site-directed mutagenesis will be used to identify DT protein residues critical for antibody binding. The information will then guide and validate the computational models. By determining the binding mode of different antibodies we hope to understand why the best binders are not the best neutralizers and what their mechanism of action is. Antibodies might prevent binding of DT to its surface receptor, for instance, or they might block the conformational change required for the activation of DT and subsequent toxicity. In addition, we would like to understand the structural determinants responsible for the elevated binding affinity of these antibodies (sub-nanomolar binding constant according to preliminary data), which would further our understanding of the general principles of protein interactions.

Structural basis for enhancement of the CXCL12 cellular activity by HMGB1 protein

Elsa Livoti, Mattia Pedotti and Luca Varani

Chemokines are a family of structurally homologues proteins that stimulate cellular movement and migration, responsible for leukocyte trafficking and homing in inflammation and other pathological conditions. The activity of individual chemokines is well characterized but much less is known about the activity of chemokines in combination to other proteins. We refer the reader to the pages of Uguccioni's and Thelen's research group for further details. In particular, Uguccioni and co-workers have shown that the cellular activity of the chemokine CXCL12 (promoting monocyte migration) is increased in the presence of another protein called HMGB1. Here we aim first of all to confirm the interaction of CXCL12 and HMGB1 with NMR spectroscopy and Surface Plasmon Resonance, and then to characterize the structure of the complex as well as its biophysical properties. NMR chemical shift mapping, an experimental technique that can identify the protein residues involved in intermolecular interfaces, will be used to characterize the binding site of HMGB1 on CXCL12 and vice versa. This information will then guide and validate computational docking studies aimed at elucidating the three-dimensional structure of the CXCL12-HMGB1 complex. Finally, we would analyze the obtained structure to identify protein residues critical for the interaction and modify them with the aim of either disrupting or improving such interaction, in order to validate our computational results. Knowing the HMGB1-CXCL12 interface we might also try to design a new molecule (peptide) mimicking the region of HMGB1 necessary to interact with CXCL12 and enhance its cellular function, but this must be considered a highly risky objective.

This work is done in collaboration with Mariagrazia Uguccioni, IRB

* **Schiraldi M. et al.** J Exp Med. 2012, 209:551-63.
- Highlights, Nat. Rev. Immunol. 2012, 12: 232.

HMGB1 binding affects the region of CXCL12 required for efficient triggering of its receptor

The individual structures of CXCL12 and HMGB1 are known, what is still missing is structural information on their complex, which we aim to provide with this research project. At the time of writing we have successfully carried out NMR mapping experiments confirming that CXCL12 binds to HMGB1. NMR mapping exploits the fact that the NMR signal is exquisitively sensitive to the local chemical environment; when a protein (in our example) binds to another, the chemical environment of residues at the binding interface changes, as does signal arising from those residues. By comparing the NMR spectrum of CXCL12 free and in complex with HMGB1 we were able to deduce which residues have a different signal and thus are involved in intermolecular complex formation. These preliminary results suggest that HMGB1 binding induces a structural change in CXCL12, activating a conformation capable of more efficient triggering of the CXCL12 receptor which, in turn, results in an increased cellular effect. The next step is to perform NMR mapping on HMGB1 in order to define its residues affected by CXCL12. Having information on the binding interface of both partners we shall be able to use it to validate the computational experiments aimed at determining the structure of the complex. In a second objective we would like to determine the amount of flexibility inherent in the structure of CXCL12. It is known, in fact, that the N-terminus of CXCL12 rapidly exchanges between different conformations. Our preliminary data suggest that the flexibility may be reduced upon binding to HMGB1, which might fix CXCL12 in a specific conformation. If this hypothesis is correct this "conformational selection" would favour the interaction between CXCL12 and its cellular receptor in two ways: i) by presenting CXCL12 in the conformation necessary for interaction with the receptor, thus facilitating interaction; ii) by lowering the entropic costs involved in receptor binding, since HMGB1, and not the receptor, would pay the energetic price of diminished flexibility at the N-terminus of CXCL12 (Figure 3).

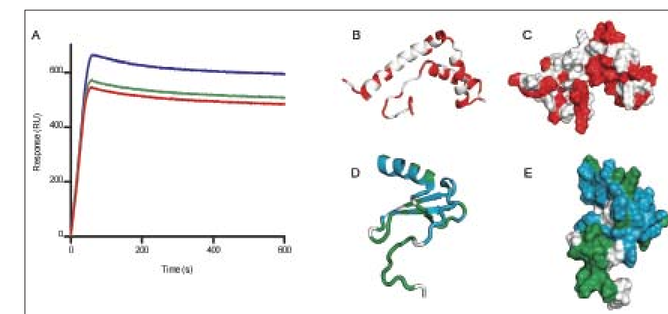

Figure 3

(A) SPR sensograms of the interaction between HMGB1 and CXCL12 in presence of increasing concentrations of glycyrrhizin. HMGB1 was immobilized on the sensor surface according to standard techniques, CXCL12 (500 nM) was then passed over the surface. CXCL12 (blue) shows a typical response curve with the signal increasing while the protein is flowed over the surface and decreasing after it stops flowing. In the presence of 500 nM glycyrrhizin (green) the signal intensity decreases, and decreases further in the presence of 5 μM glycyrrhizin (red). This is indicative of decreased CXCL12 binding. Cartoon (B) and surface (C) representation of BoxB: residues whose NMR signal is affected by CXCL12 binding are in red. (D and E). CXCL12 residues affected by HMGB1 binding are colored on the cartoon (D) and surface (E) representation of CXCL12. Residues affected by binding of either BoxA or BoxB are in blue; residues that are affected only upon addition of full HMGB1 are in green.

Funding

SVRI
Computer assisted vaccine design
2009-2011

CSCS
Prediction and characterization of intermolecular interactions in human diseases
2007-2011

European Union
Bio-NMR - HMGB1 binds to CXCL12 and increases its cellular activity: a structural investigation.
(BIO-NMR-00066) / 2011

MRC Biomedical NMR Centre
Characterization of antibody-protein interaction in Dengue virus
2011

Collaborations

CSCS, Swiss Supercomputer Center
Manno (CH)

NMR Centre at MRC-NIMR
Mill Hill, London (UK)

University of Frankfurt
Frankfurt (DE)

Luigi Calzolai
European Union Joint Research Center
Ispra (IT)

Publications

Computational docking of antibody-antigen complexes, opportunities and pitfalls illustrated by influenza hemagglutinin.
Pedotti, M., L. Simonelli, E. Livoti and L. Varani.
Int J Mol Sci. 2011; 12:226-51.

HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4.
Schiraldi, M., A. Raucci, L. Martínez Muñoz, E. Livoti, B. Celona, E. Venereau, T. Apuzzo, F. De Marchis, M. Pedotti, A. Bachi, M. Thelen, L. Varani, M. Mellado, A. Proudfoot, M. Emilio Bianchi and M. Uguccioni.
J Exp Med. 2012; 209:551-63.

Lectures and Seminars

Ospedale San Gerardo Monza
Monza (IT) / 02.2011

Universita' Svizzera Italiana, USI
Lugano (CH) / 03.2011

Lausanne Biomolecular Modelling Seminars, SIB
Lausanne (CH) / 06.2011

CECAM workshop
Lugano (CH) / 09.2011

CORE FACILITIES
SECTION 2

INDEX SECTION 2 – CORE FACILITIES

99 **Imaging Facility**
100 **Gene Expression and Protein Production Facility**

The Imaging facility is central to most of the research projects and includes a Flow cytometry lab and a Microscopy lab. The Flow cytometry lab that is run by David Jarrossay who takes care of the cell sorting, maintenance of the equipment, instruction and advice to the new operators, in addition to performing his own research. Erica Montani has been recruited in 2011 to run the Microscopy lab. She has experience in confocal microscopy and high content cellular analysis.

Flow cytometry lab

The Flow cytometry lab provides investigators with equipment and support for cell sorting (separation), acquisition, and analysis of flow cytometric data with a variety of state-of-the-art multicolor flow cytometry instruments.

Cell sorting is performed via a FACSAria III sorter equipped with four lasers (488, 561, 640 and 405 nm excitation wavelengths) and 15 fluorescence channels detection. It can perform high speed sorting (up to 20,000 events/sec) with high-purity (up to 99%). The Flow cytometry lab is equipped with an advanced benchtop analyzers BDLSR Fortessa equipped with four lasers (488, 561, 640 and 405 nm excitation wavelengths) and 16 fluorescence channels. The lab also offers to researchers one FACSCantoII analyser (three lasers-eight colors), a FACSCanto I (two lasers-six colors), a FACSCalibur (two lasers-four colors) and a FACSArray plate based flow cytometer (two lasers-four channels). Both FACSCanto are equipped with HTS for high throughput screening for 96 and 384 wells plates. The lab is also equipped with a Flexmap 3D instrument for bead-based multiplexing for up to 500 analytes per well on a 96 or 384 well plate format.

The staff provides cell sorting on BD FACSAria III, individual training on bench top analysers, maintenance of all instruments and assistance with experimental design, data analysis and troubleshooting.

Microscopy lab

The main Microscopy lab comprises a suite of confocal, high-content imaging system for image acquisition and analysis, wide-field upright and inverted microscopes:

- Confocal microscope. Leica sp5, equipped with new generation detectors (HyD detector) and high resolution objective (100x, NA 1.44)

- High-content imaging system. BD Pathway 855, wide-field or confocal automated microscope suitable for screening. Equipped with Twister II Plate Handler (Caliper) allows to acquire and analyze up to 35 plates.
- Wide-field microscopes. Nikon Eclipse upright and inverted microscopes. A Zeiss axiovert 200 inverted microscope set for calcium measurement experiments.

With these systems we are able to perform most of the procedures for cells and tissues imaging including FRET, FRAP and live cell imaging. We also offer support for sample preparation, image analysis, deconvolution and 3D reconstruction thanks to a wide range of software such as MetaMorph (Molecular Devices), Imaris (Bitplane), ImageJ and CellProfiler.

Gene Expression and Protein Production Facility (GEPP)

The IRB has developed relevant competence in the field of protein-protein and antigen-antibody interaction applied to the study of inflammatory and infectious diseases.

The Gene Expression and Protein Production facility (GEPP), directed by Laurent Perez, helps IRB researchers with cellular bioche-mistry, protein expression and production but also develops and evaluates new techniques for protein production and purification. Assistance and information are offered to all IRB research laboratories. The facility is equipped for small to medium scale purification from cell free system, bacteria, insect and mammalian cell lines. It is currently specializing in the use of the baculovirus expression system and mammalian expression, especially for production of multimeric protein complexes. Depending upon the level of expression of a given protein, quantity ranging from milligrams to grams can be produced using resources at the facility. Finally, the facility is centralizing stocks of a large number of expression vectors for insect and mammalian cells. Equipment available in the GEPP facility: ÄKTA purifier, Millipore tangential flow filtration system and a ProteOn XPR36 machine. This facility is enhanced by collaboration with the Swiss Supercomputer Center located in Ticino as well as with several international institutions.

PhD PROGRAMME

SECTION 3

INDEX SECTION 3 – PhD PROGRAMME

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104 Chiara Borsotti - The RAG2$^{-/-}\gamma c^{-/-}$ humanized mouse model: an effective tool to study "in vivo" the human adaptive immune system development and to preclinically test the targeting of its cells.
105 Michela Frascoli - ATP as a signaling molecule in haematopoietic stem cell and early T cell development
106 Francesca Ronchi - On the role of IL-1β in T cell-mediated immunopathology
107 Milena Schiraldi - The alarmin HMGB1 enhances CXCR4-induced activities by binding CXCL12

Tiziana Apuzzo

Characterization of eIF2B as a putative novel CXCR4 effector branch

Supervisor: Marcus Thelen // Co-referees: Mario Tschan
Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland

Schiraldi M. et al. *
J Exp Med. 2012, 209:551-63.
- Highlights, Nat. Rev. Immunol. 2012, 12: 232.

Volpe S. et al. *
PLoS One. 2012, 7:e37208.

Chemokine receptors belong to the rhodopsin-like subfamily of heptahelical G-protein coupled receptors and share the ability to mediate leukocyte migration. The ubiquitously expressed chemokine receptor CXCR4 was shown to have, in addition to its function on leukocytes, a critical role during embryogenesis and hematopoiesis. The marked appearance on many invasive cancer cells 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. CXCR4 further distinguishes from other chemokine receptors through its signaling properties and functional responsiveness. It is conceivable that CXCR4, in addition to Gi-coupling, interacts with other proteins, which regulate the characteristic responses of the receptor. The cytoplasmic surface of a chemokine receptor consists of the C-terminus and 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. Immunoprecipitation of CXCR4 under mild detergent conditions which preserves its native conformation and the association with downstream effectors. Mass-spectrometry was used to unveil several, unreported interactions of CXCR4 with cytosolic proteins. Among the proteins identified were SHBP1 and eIF2B.
SH2BP or p150 TSP = *T*PR-containing, SH2-binding *p*hosphoprotein, was reported as a nuclear protein. However, our data indicate that SH2BP also associates with CXCR4 at the plasma membrane. In cell fractionation experiments the localization of SH2BP in different subcellular compartments was determined. The specific association of SH2BP with CXCR4 was further confirmed in pull down experiments using biotinylated CXCL12 (ligand of CXCR4).
The translation initiation factor eIF2B consists of 5 subunits which were all detected as associated proteins in CXCR4 immunoprecipitates. The finding was confirmed in different experimental settings and led to the hypothesis that CXCR4 can induce protein synthesis from pre-existing mRNA near the plasma membrane. In line with the hypothesis eIF2B was found to dissociate from CXCR4 upon receptor activation.

Chiara Borsotti

The RAG2$^{-/-}\gamma_c^{-/-}$ humanized mouse model: an effective tool to study in vivo the human adaptive immune system development and to preclinically test the targeting of its cells.

Supervisor: Markus Manz // Co-referee: Antonio Lanzavecchia
PhD Program in Molecular Medicine, Vita Salute San Raffaele University, Milan, Italy

Immunodeficient mice reconstituted with human hematopoietic stem and progenitor cells develop a human hemato-lymphoid system (HHLS) and therefore they have the potential to advance significantly biomedical and translational research. Even though the development and the function of several human cell lineages are suboptimal, those mice have been successfully used in many studies for a better understanding of the differentiation potential of human immune populations, for the analysis of human pathogens and for vaccine development.

As first we sought to characterize in details the B cell compartment in the human CD34$^+$ cell injected RAG2$^{-/-}\gamma_c^{-/-}$ (hu-RAG2$^{-/-}\gamma_c^{-/-}$) mouse model. We compared the human B cell development between human IL6 knock-in and mouse IL6 hu-RAG2$^{-/-}\gamma_c^{-/-}$ mice. The presence of the human IL6 gene led to a better maintenance of the human cells in periphery. We observed a partial block in the B cell maturation process with an accumulation of immature/transitional cells in the spleen of the mice and the presence of a consistent CD5$^+$ population. Nevertheless we detected memory B cells and plasma cells (PCs) in the engrafted mice as well as human immunoglobulins (Igs).

Moreover we used the RAG2$^{-/-}\gamma_c^{-/-}$ mouse to test the capacity of human T cell progenitors, obtained *in vitro* from mobilized CD34$^+$ cells, to home and fully mature in the thymus. Indeed these cultured cells were able to specifically migrate in the thymus and to become CD3 and TCRα/β positive passing through the physiological CD4$^+$CD8$^+$ double positive stage faster than the non-cultured CD34+ cells.

Lastly we investigated the depleting activity of two anti-human monoclonal antibodies (mAbs), an α-CD20 and an α-CD4, respectively on B and CD4$^+$ T cells developed in the hu-RAG2$^{-/-}\gamma_c^{-/-}$ mice. The injections of the mAbs led to a faithfully elimination of the target cells and allowed the study of the treatment effect even in organs difficult to analyze in humans.

Overall our findings highlight the feasibility and the importance of the RAG2$^{-/-}\gamma_c^{-/-}$ humanized mouse as a tool to investigate the development of different populations of the human adaptive immune system and as a good preclinical model to carefully assess the safety and the efficacy of anti-human mAb therapies.

* ***Legrand N. et al.*** *Cell Host Microbe 2009, 6: 5-9.*

* ***Meek B. et al.*** *Blood 2010, 115: 261-4*

* ***Strowig T. et al.*** *Proc Natl Acad Sci USA 2011, 108: 13218-23.*

Michela Frascoli

ATP as a signaling molecule in haematopoietic stem cell and early T cell development

Supervisor: Fabio Grassi // Co-referees: Clemens A. Dahinden and Jens Stein
Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland

Haematopoietic stem cells (HSCs) express Toll-like receptors (TLRs) to respond to Pathogen-Associated Molecular Patterns (PAMPs) as well as receptors for Damage-Associated Molecular Patterns (DAMPs). Extracellular ATP belongs to the family of DAMPs and it can promote several immune cells functions. Mice suffering from chronic inflammation are characterized by expansion of HSCs pool and altered representation of haematopoietic progenitors in the bone marrow. *In vitro* and *in vivo* experiments with periodate-oxidized ATP (oATP), a P2X receptors antagonist, demonstrated the involvement of P2X receptors in HSCs proliferation induced by TLRs stimulation. These results point to a function for extracellular ATP as a regulator of HSCs population size.

In immature T cells, lineage choice is dictated by T cell receptor (TCR) signal strength with increasing strength resulting in induction of the γδ differentiation program. Indeed, artificial reduction of γδ TCR signalling was shown to divert γδ TCR expressing cells toward the αβ fate. We investigated whether pericellular ATP were controlling signal strength in thymocytes as observed in mature T cells. Analysis of transcriptional regulation of purinergic receptors in thymocyte subsets by real time (RT)-PCR revealed dominant expression of P2X4 and P2X7 receptors in the precursors of the αβ and γδ lineage. Interestingly, expression of P2X7 receptor is selectively increased in immature γδ CD25$^+$ cells. Analogously to mature T cells, these cells released ATP following TCR stimulation. Fetal thymus organ cultures (FTOC) of *p2rx7* knock-out thymi revealed the generation of aberrant CD4$^+$8$^+$ cells (e.g. αβ committed) expressing γδ TCR. This phenomenon correlated with impaired ERK phosphorylation and downregulation of γδ-specific Egr transcription factors. These results indicate that γδTCR expression in immature thymocytes is coupled to increased ATP synthesis and release as compared to pre-TCR expression. This feature, together with enhanced P2X7 receptor expression and signaling in γδ$^+$CD25$^+$ cells, delineates an autocrine loop, which influences γδ lineage choice.

Traggia E. et al. * *PLoS One 2010, 5:e11262.*

Casati A., Frascoli M. et al. * *PLoS One 2012, under revision*

Schenk U. et al. * *Sci Signal. 2011, 4:ra12.*

Francesca Ronchi

On the role of IL-1β in T cell-mediated immunopathology

Supervisor: Federica Sallusto // Co-referee: Vincenzo Barnaba
PhD Program in Molecular Medicine, Vita Salute San Raffaele University, Milan, Italy

IL-1β is a pleiotropic cytokine that plays a role in several inflammatory disorders in humans and in experimental animal models, including mouse experimental autoimmune encephalomyelitis (EAE). IL-1β is produced after cleavage of pro-IL-1β by interleukin-1 converting enzyme (caspase-1), which in turn is activated by a complex of proteins called inflammasome. This cytokine has been shown to be required for differentiation of human Th17 cells, and more recently, to trigger differentiation of mouse inflammatory Th17 cells characterized by co-expression of IL-17 and IFNγ. In this study we reported that mice deficient for IL-1β or for a component of the inflammasome (the apoptosis-associated speck-like protein containing a caspase recruitment domain, also known as ASC) did not develop EAE following immunization with myelin oligodendrocyte glycoprotein (MOG) in complete Freund's adjuvant (CFA) and pertussis toxin (PT). Autoreactive T cells were primed in wild-type (WT), IL-1β$^{-/-}$ and ASC$^{-/-}$ mice. However, while in WT mice T cells proliferated extensively and acquired the capacity to produce inflammatory cytokines, such as IL-17, IL-22, IFNγ, and GM-CSF, in IL-1β$^{-/-}$ and ASC$^{-/-}$ mice the cells expanded poorly and showed reduced capacity to produce simultaneously several inflammatory cytokines, in particular GM-CSF. Interestingly, induction of polyfunctional (IL-17^{+} IL-22^{+} IFNγ^{+} GM-CSF^{+}) T cells in WT mice was dependent on the presence of PT at the time of immunization. PT was found to rapidly induce IL-1β secretion by CD11c^{+} and Gr1^{+} myeloid cells in secondary lymphoid organs *in vivo*, and by bone-marrow-derived dendritic cells *in vitro*. Moreover, in mice where Gr1^{+} myeloid cells were depleted IL-1β production was not induced by PT and priming of polyfunctional T cells was impaired. Taking together these data supported the notion that the disease-inducing effect of PT was due to its ability to induce recruitment of Gr1^{+} myeloid cells, production of IL-1β and differentiation of pathogenic polyfunctional T cells.

* *Vigne S. et al.* Blood 2011, 118:5813-23.

Milena Schiraldi

The alarmin HMGB1 enhances CXCR4-induced activities by binding CXCL12

Supervisor: Mariagrazia Uguccioni // Co-referee: Jürg Tschopp
PhD Program in Science de la vie, University of Lausanne, Switzerland

Schiraldi M. et al. * J Exp Med. 2012, 209:551-63. - Highlights, Nat. Rev. Immunol. 2012, 12: 232.

Gouwy M. et al. * Immunology Letters. 2012, in press.

Venetz D. et al. * Int J of Cancer. 2010, 127(10):2300-12

Kuscher K. et al. * Eur J Immunol. 2009, 39(4):1118-28

After tissue damage, inflammatory cells infiltrate the tissue and release proinflammatory cytokines. HMGB1 (high mobility group box 1), a nuclear protein released by necrotic and severely stressed cells, promotes cytokine release via its interaction with the TLR4 (Toll-like receptor 4) receptor and cell migration via an unknown mechanism. We investigate the mechanisms of HMGB1-induced recruitment of inflammatory cells in relation to the chemokine CXCL12. HMGB1 and CXCL12 form a heterocomplex, which we characterized by nuclear magnetic resonance and surface plasmon resonance, that acts exclusively through CXCR4 and not through other HMGB1 receptors. Fluorescence resonance energy transfer data show that the HMGB1–CXCL12 heterocomplex promotes different conformational rearrangements of CXCR4 from that of CXCL12 alone. Mononuclear cell recruitment in vivo into air pouches and injured muscles depends on the heterocomplex and is inhibited by AMD3100, the CXCR4 antagonist, and glycyrrhizin, the sweet tasting compound of the liquorice root, known to interact with HMGB1 and ,as we showed, blocking the heterocomplex-induced migration *in vitro* and *in vivo*. Thus, inflammatory cell recruitment is sustained in the presence of the heterocomplex and molecules known to interfere with HMGB1-CXCL12 complex formation, such as glycyrrhizin act as inhibitory of cell recruitment. This study is therefore important for the understanding of HMGB1-induced migration in diseases, like rheumatoid arthritis and tumor, in which the heterocomplex HMGB1-CXCL12 can be specifically inhibited.

PhD LECTURE COURSE & SEMINARS

SECTION 4

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111 PhD Lecture course 2011
112 Seminar Programme 2011

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

PhD Lecture Course 2011

Gioacchino Natoli
"Transcriptional control of inflammation"
Campus IFOM-IEO, Milan (IT) / 03.11.10

Mario Roederer
"Progenitor memory T cells"
Vaccine Research Center, NIAID, NIH, Bethesda, MD (US) / 14.12.10

Amanda Fisher
"Stem cells and reprogramming"
MRC Clinical Sciences Centre, Imperial College London, London (UK) / 01.03.11

Hiroshi Takayanagi
"Osteoimmunology"
Tokyo Medical and Dental University, Tokyo (JP) / 21.03.11

Frances Balkwill
"Cancer and inflammation"
Centre for Cancer and Inflammation, Barts Cancer Institute, Queen Mary University of London, London (UK) / 25.03.11

Walter Reith
"Regulation and function of antigen presentation"
University of Geneva Medical School, Geneva (CH) / 13.04.11

Arturo Zychlinsky
"Neutrophils in microbial infections"
Department of Cellular Microbiology, Max Planck Institute for Infection Biology, Berlin (DE) / 05.05.11

Sidonia Fagarasan
"Dynamic interactions between bacteria and immune cells in the gut"
Research Center for Allergy and Immunology, RIKEN Yokohama Institute, Kanagawa (JP) / 16.06.11

Judy Lieberman
"Cell death activated by killer lymphocytes"
Immune Disease Institute, Harvard Medical School, Boston (US) / 08.07.11

Seminar Programme 2011

Gioacchino Natoli
"The macrophage epigenome and the control of inflammatory gene expression"
Campus IFOM-IEO, Milan (IT) 03.11.10

Mario Roederer
"Identification of T memory stem cells: Dynamics under stress"
Vaccine Research Center, NIAID, NIH, Bethesda, MD (US) 14.12.10

Bill Schief
"Structure-based immunogen design"
University of Washington, Seattle (US) 20.01.2011

Pietro Speziale
"A survey on Staphylococcus aureus surface-expressed and secreted proteins as potential components of a multivalent vaccine"
Università di Pavia, Pavia (IT) 22.02.2011

Amanda Fisher
"Stem cells and reprogramming"
MRC Clinical Sciences Centre, Imperial College London, London (UK) 01.03.11

Hiroshi Takayanagi
"Bone destruction in arthritis and osteoimmunology"
Tokyo Medical and Dental University, Tokyo (JP) 21.03.11

Frances Balkwill
"Inflammatory cytokines and autocrine tumour-promoting networks"
Centre for Cancer and Inflammation, Barts Cancer Institute, Queen Mary University of London, London (UK) 25.03.11

Fausto Baldanti
"Emerging and re-emerging respiratory viruses"
Fondazione IRCCS Policlinico San Matteo, Pavia (IT) 05.04.2011

Walter Reith
"Critical role of MHC class II mediated antigen presentation in homeostasis of the medulla in the postnatal thymus"
University of Geneva Medical School, Geneva (CH) 13.04.11

Eloísa Yuste
"Broadly Cross-Neutralizing Antibodies in HIV-1 Patients with Undetectable Viremia"
IDIBAPS – Faculty of Medicine, Barcelona (ES) 28.04.2011

Laurent-Hervé Perez
"Biochemistry to investigate host-pathogen interactions"
Philip Morris Products S.A., Neuchatel (CH) 29.04.2011

Arturo Zychlinsky
"NETs – from infection to autoimmunity"
Department of Cellular Microbiology, Max Planck Institute for Infection Biology, Berlin (DE) 05.05.11

Andrea Cavalli
"Multi-scale modeling of proteins and protein complexes"
University of Cambridge, Cambridge (UK) 11.05.2011

Dirk Bakowies
"Molecular Dynamics Simulations - What do they tell us and how do we understand them?"
ETH Zurich, Zurich (CH) 30.05.2011

Hanna Sundström
"Structural and Functional Studies of Antimicrobial Proteins, Voltage-gated Ion Channels and Transmembrane Receptors"
Paul Scherrer Institut, Villigen (CH) 31.05.11

Fabio Sessa
"Structural and Biochemical analysis of the Proteasome Lid Complex"
Institute for Research in Biomedicine and Institut de Biologia Molecular de Barcelona (CSIC), Barcelona (ES) 09.06.2011

Sidonia Fagarasan
"T cell-independent and T cell-dependent lgA synthesis in the gut"
Research Center for Allergy and Immunology, RIKEN Yokohama Institute, Kanagawa (JP) 16.06.11

Ueli Aebi
"Novel peptide nanoparticles: their use for synthetic vaccine design, targeted drug delivery and bio-imaging"
M.E. Müller Institute for Structural Biology Biozentrum, University of Basel, Basel (CH) 04.07.2011

Umberto Bertazzoni
"Viral carcinogenesis and oncogenic proteins Tax-1 and Tax-2 of human retrovirus HTLV"
Faculty of Medicine, University of Verona, Verona (IT) 05.07.2011

Peter Kolosov
"The regions of the N-terminal domain of translation termination factor responsible for stop-codons recognition in mRNA"
Center of Bioengineering RAS, Moscow (RU) 05.07.2011

Oliver Frey
"Arthritis induced by systemic autoimmunity: T cells and beyond"
University Hospital Jena, Institute of Clinical Chemistry and Laboratory, Jena (DE) 07.07.2011

Judy Lieberman
"Delivering the kiss of death: A new model for perforin"
Immune Disease Institute, Harvard Medical School, Boston (US) 08.07.11

Samuele Notarbartolo
"Unraveling the role of the histone demethylase Kdm5b/Jarid1b in macrophages"
European Institute of Oncology (IEO), Milan (IT) 12.07.2011

Lars Knoch
"TALEN®: Innovative genome customization tools to knock out genes and introduce modifications in any gene, at any genomic position, in any cell type"
Cellectis bioresearch, Romainville (FR) 19.08.2011

Stephan Urban
"Exploiting the liver tropism of the human Hepatitis B virus (HBV): From HBV Entry Inhibition to hepatocyte-specific Drug Targeting"
Otto-Meyer-Zentrum, University of Heidelberg (DE) 09.09.2011

Derya Unutmaz
"Functional definition and flexibility of human Tregs and Th17 cells"
NYU Langone Medical Center, New York (US) 19.09.2011

Antonio Uccelli
"Mesenchymal stem cells: a new cell therapy for the treatment of autoimmunity"
Ophthalmology and Genetics – University of Genoa (IT) 04.10.2011

Philip M. Murphy
"Development of Mozobil as a Potential Therapeutic in WHIM Syndrome"
National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD (US) 07.10.2011

Gisou Van Der Goot
"Towards dissecting a cytosolic ER folding-quality control machinery"
Global Health Institute, Ecole Polytechnique Fédérale of Lausanne, Lausanne (CH) 12.10.2011

Marco E. Bianchi
"HMGB1 is both a Damage Associated Molecular Pattern and a cell-autonomous effector of stress response"
San Raffaele University, Milan (IT) 18.10.2011

Pramod K. Srivastava
"New vistas in cancer immunity"
Carole and Ray Neag Cancer Center, University of Connecticut School of Medicine, Farmington, CT (US) 24.10.2011

Jakob Loschko
"Antigen targeting to plasmacytoid dendritic cells - induction of tolerance or immunity"
Technical University of Munich, Munich (DE) 31.10.2011

Marina de Bernard
"Modulation of the host immune response by Helicobacter pylori Neutrophil Activating Protein and its possible applications in therapy"
University of Padua, Venetian Institute of Molecular Medicine, Padua (IT) 08.11.2011

Annalisa Pastore
"Polyglutamine and Neurodegeneration"
The National Institute for Medical Research, London (UK) 16.12.2011

PEOPLE & FINANCES
SECTION 5

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IRB PERSONNEL

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Mäxi Stiftung

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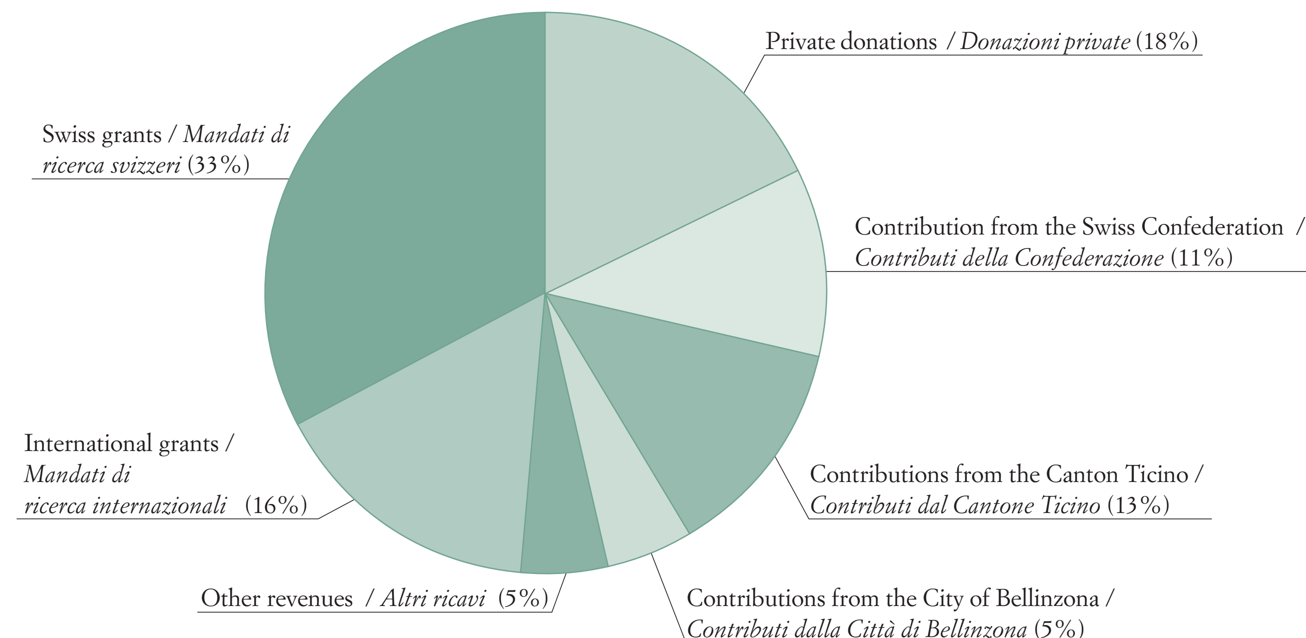
Others

Financial Data 2011 / *Dati finanziari 2011*

The continuous positive development of the research activities has allowed the Institute to grow in an important way during 2011 with a final balance surpassing CHF 15.5 million, an increase by more than 15% in comparison to 2010.
Funding from research projects (Swiss National Science Foundation, European Union, Swiss and International private Foundations...) have risen even more markedly, passing from CHF 5.7 million to CHF 7.6 million (up to 32,7% increase!) and financed nearly half of the entire budget of the Institute.

La continua positiva evoluzione dell'attività di ricerca ha permesso all'Istituto di crescere nel corso del 2011 in maniera importante, superando a consuntivo i CHF 15,5 milioni di costi, con un incremento rispetto all'anno 2010 di oltre il 15%.
I finanziamenti provenienti da progetti di ricerca (Fondo Nazionale Svizzero per la Ricerca, Comunità Europea, Fondazioni private svizzere e internazionali, …) sono aumentati in maniera ancora più marcata, passando da CHF 5,7 milioni a CHF 7.6 milioni (aumento del 32,7%!) e hanno contribuito a finanziare quasi la metà del budget dell'intero Istituto.

Funding by source 2011 / *Contributi per fonte 2011*



Balance Sheet as 31 of December 2011 (In Swiss Francs) / *Bilancio al 31 dicembre 2011 (in Franchi Svizzeri)*

ASSETS / *ATTIVO*	31.12.2011	31.12.2010
1. Liquidity / *Liquidità*	11'379'967	15'866'560
2. Various Receivables / *Crediti diversi*	1'844'898	910'550
3. Temporary Receivables / *Transitori attivi*	1'008'699	524'584
Current Assets / *Attivo circolante*	14'233'563	17'301'694
4. Participations / *Partecipazioni*	12'500	12'500
5. Buildings / *Immobili*	4'567'440	4'841'000
6. Furnishing & Equipment / *Attrezzature*	880'000	1'300'000
Fixed Assets / *Attivo fisso*	5'459'940	6'153'500
Total Assets / *Totale attivo*	19'693'503	23'455'194

LIABILITIES / *PASSIVO*	31.12.2011	31.12.2010
1. Debt for Delivery and Services / *Debiti per forniture e prestazioni*	676'066	708'379
2. Accruals / *Accantonamenti e transitori passivi*	1'861'317	1'742'994
3. Funds for Research Projects / *Fondi progetti di ricerca*	2'626'611	3'886'987
4. Funds for Laboratories / *Fondi dei laboratori*	2'248'717	1'864'259
5. Various Funds / *Fondi diversi*	2'261'514	4'276'765
Current Liabilities / *Capitale estraneo a breve termine*	9'674'225	12'479'384
6. Long Term Loans / *Prestiti a lungo termine*	2'800'000	3'800'000
Long Term Liabilities / *Capitale estraneo a lungo termine*	2'800'000	3'800'000
7. Capital Resources / *Capitale proprio*	7'175'810	7'017'001
8. Annual Result / *Risultato d'esercizio*	43'468	158'809
Equity of the Foundation / *Capitale della Fondazione*	7'219'278	7'175'810
Total Liabilities / *Totale passivo*	19'693'503	23'455'194

Profit and Loss Account for the year 2011 (In Swiss Francs) / *Conto economico esercizio 2011 (in Franchi svizzeri)*

COSTS / *COSTI*	2011	2010
1. Personnel Costs / *Costi del personale*	5'996'271	5'736'888
2. Consumables / *Fabbisogno medico*	2'169'745	1'701'981
3. Maintenance of Buildings and Equipment / *Manutenzione immobili e attrezzature*	707'344	711'466
4. Investments / *Investimenti*	2'113'084	1'189'377
5. Amortizations / *Ammortamenti*	999'638	1'003'826
6. Rent and Related Costs / *Affiti e altri costi dei locali*	1'342'705	1'284'692
7. Administrative Costs and Various / *Costi generali amministrativi e diversi*	1'144'875	935'982
8. Travels, Congresses and Guests / *Trasferte, congressi, viaggi e ospiti*	290'049	275'792
9. Financial charges / *Oneri finanziari*	30'949	30'975
10. Various Costs for Research / *Altri costi di ricerca*	719'345	554'438
Total Costs / *Totale costi*	15'514'005	13'425'417

REVENUES / *RICAVI*	2011	2010
1. Contributions from the Confederation / *Contributi Confederazione*	1'729'000	1'550'000
2. Contribution from the Canton Ticino / *Contributi Canton Ticino*	2'000'000	2'000'000
3. Contribution from the City of Bellinzona / *Contributi Città di Bellinzona*	720'000	720'000
4. Contributions from the Helmut Horten Foundation / *Contributi Fondazione Helmut Horten*	1'768'000	1'768'000
5. Other Contributions / *Altri Contributi*	1'001'433	1'187'879
6. Research Projects / *Progetti di ricerca*	7'562'556	5'700'539
7. Other Revenues / *Altri ricavi*	776'483	657'808
Total Revenues / *Totale ricavi*	15'557'473	13'584'226

ANNUAL RESULT / RISULTATO D'ESERCIZIO	**43'468**	**158'809**

PUBLICATIONS

SECTION 6

INDEX SECTION 6 – PUBLICATIONS

125 Peer Reviewed Publications
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Publications are numbered progressively since the founding of the IRB in 2000

Peer Reviewed Publications from January 2011

272. **Human antibodies against dengue enhance dengue viral infectivity without suppressing type I interferon secretion in primary human monocytes.**
Kou, Z., J. Y. Lim, M. Beltramello, M. Quinn, H. Chen, S. N. Liu, L. Martnez-Sobrido, M. S. Diamond, J. J. Schlesinger, A. de Silva, F. Sallusto, and X. Jin.
Virology. 2011; 410:240-247.

273. **Strain-transcending Fc-dependent killing of plasmodium falciparum by merozoite surface protein 2 allele-specific human antibodies.**
Stubbs, J., S. Olugbile, B. Saidou, J. Simpore, G. Corradin, and A. Lanzavecchia.
Infect Immun. 2011; 79:1143-1152.

274. **Human thrombopoietin knockin mice efficiently support human hematopoiesis in vivo.**
Rongvaux, A., T. Willinger, H. Takizawa, C. Rathinam, W. Auerbach, A.J. Murphy, D.M. Valenzuela, G.D. Yancopoulos, E.E. Eynon, S. Stevens, M.G. Manz, and R.A. Flavell.
Proc Natl Acad Sci U S A. 2011. 108:2378-2383.

275. **CSF knock-in mice support human alveolar macrophage development and human immune responses in the lung.**
Willinger, T., A. Rongvaux, H. Takizawa, G.D. Yancopoulos, D.M. Valenzuela, A.J. Murphy, W. Auerbach, E.E. Eynon, S. Stevens, M.G. Manz, and R.A. Flavell. Human IL-3/GM-
Proc Natl Acad Sci U S A. 2011. 108:2390-2395.

276. **Malectin participates in a backup glycoprotein quality control pathway in the mammalian ER.**
Galli, C., R. Bernasconi, T. Solda, V. Calanca, and M. Molinari.
PLoS One. 2011; 6:e16304.

277. **Dynamic variation in cycling of hematopoietic stem cells in steady state and inflammation.**
Takizawa, H., R.R. Regoes, C.S. Boddupalli, S. Bonhoeffer, and M.G. Manz.
J Exp Med. 2011. 208:273-284.

278. **Computational docking of antibody-antigen complexes, opportunities and pitfalls illustrated by influenza hemagglutinin.**
Pedotti, M., L. Simonelli, E. Livoti, and L. Varani.
Int J Mol Sci. 2011; 12:226-251.

279. **Improved innate and adaptive immunostimulation by genetically modified HIV-1 protein expressing NYVAC vectors.**
Quakkelaar, E. D., A. Redeker, E. K. Haddad, A. Harari, S. M. McCaughey, T. Duhen, A. Filali-Mouhim, J. P. Goulet, N. M. Loof, F. Ossendorp, B. Perdiguero, P. Heinen, C. E. Gomez, K. V. Kibler, D. M. Koelle, R. P. Sekaly, F. Sallusto, A. Lanzavecchia, G. Pantaleo, M. Esteban, J. Tartaglia, B. L. Jacobs, and C. J. Melief.
PLoS One. 2011; 6:e16819.

280. **Cell-autonomous regulation of hematopoietic stem cell cycling activity by ATP.**
Casati, A., M. Frascoli, E. Traggiai, M. Proietti, U. Schenk, and F. Grassi.
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