Università della Svizzera italiana

Institute for Research in Biomedicine

Scientific Report 2010

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This Scientific Report covers the 2010 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

The Institute for Research in Biomedicine, which occupies two buildings, Via Vela and Via Murate, in Bellinzona, celebrated its first ten years of activity with a ceremony that took place on November 5th, 2010 at the Teatro Sociale in Bellinzona in the presence of the cantonal and communal authorities, the State Secretary for Education and Research (SER), Dr. Mauro Dell'Ambrogio, the President of the University of Italian Switzerland (USI), Prof. Piero Martinoli, some representatives from our supporting foundations, private donors as well as many other guests.
This event was a wonderful opportunity to look over our achievement and to list the progresses and advances already made since the creation of the IRB, which currently hosts 8 research groups with more than 80 employees.

In its report from February 2010, the Scientific Council of the IRB has expressed this gratifying opinion: "The Institute for Research in Biomedicine in Bellinzona continues to have an excellent worldwide reputation and should be praised for its excellence and its productivity in research. Its scientific quality is validated by their numerous publications in the field of biomedical research (more than 250 since 2000 in the highest scientific peer-reviewed international journals with an average impact factor of 11.5), by progresses in their methodological approaches and constant advances in health such as in infectious diseases, vaccination, autoimmune pathologies and associated diseases".

Ten patents were registered and licensed to the Humabs company, which transferred its headquarters from the United States to Bellinzona in 2010, occupying two research laboratories at the IRB, Via Murate.

The IRB is also a founding member of the Swiss Vaccine Research Institute (SVRI), along with the Swiss Federal Institute of Technology Lausanne (EPFL), the Faculty of Medicine of the University of Lausanne (UNIL) and the Ludwig Institute also in Lausanne. A partnership with the Swiss Federal Institute of Technology Zurich (ETHZ) was initiated in 2008 and the IRB Director, Antonio Lanzavecchia, was appointed Professor of Immunology at this prestigious institution in 2009. This partnership will permit the IRB to host doctoral and master students from the ETHZ, which in return will provide support regarding the IRB Intellectual Property issues. In April 2010, the High Council from Tessin approved the affiliation of the IRB to the University of Italian Switzerland. This affiliation will permit IRB constant financing from the Canton in forms of services, and hopefully in the near future, the recognition by USI of the IRB PhD program, which currently counts around 30 students who follow lectures given by scientific experts coming not only from Switzerland, but also from Europe, the United States and Japan. Since the IRB creation, more than 40 stu-

dents have obtained their doctoral degree at Swiss and foreign universities. Moreover, it is planned that the IRB will play an important role in the development of the "Master Medical School" at the University of Italian Switzerland.

The IRB collaborates with numerous Swiss and foreign institutions. It also shares its headquarters with the Institute of Oncology of Southern Switzerland (IOSI) and very recently with the Neurocentre from the Cantonal Hospital (EOC), and collaborates with the Institute for Computational Sciences at the University in Lugano, the Institute of Microbiology (LCM) in Bellinzona and the Cantonal Institute of Pathology in Locarno.

The IRB annual budget reaches around 14-16 Million CHF thanks to public institutions (Confederation, Canton and City) and private foundations, in particular the Helmut Horten Foundation and the Jacob Foundation but also individual sponsors.

Moreover, extremely competitive research grant applications permit the receipt of funding from agencies such as the Swiss National Science Foundation, the European Union and the National Institute of Health from the USA or the Bill & Melinda Gates Foundation and other private Swiss and foreign foundations, which contribute up to around 45% of the IRB budget.

The constant growth of the IRB and its associated institutes renders the necessary construction of a new building, for which the IRB has commissioned a feasibility study with the architect Mario Campi, who recently designed the new ETH laboratories in Höngg. For this project, the Municipality of Bellinzona has decided to concede the 13'000 m^2 surface of the ex-military campus, a decision that was accepted by the Town Council (February 28, 2011). Unfortunately, some oppositions were raised. However, we trust that the realization of such an important construction will allow us to continue to work in good conditions and to obtain further successes in the life sciences for the development of the City and of the whole Canton. In the mean time, we will continue to give our best to further improve our research work.

Finally, I would like to underline the exceptional and essential contribution from our core funding, in particular from the public institutions and from the Helmut Horten and Jacob Foundations, and thank the members of the IRB Foundation Council, the IRB Director and all the IRB personnel who contribute to the excellent collaboration in a modern, dynamic and pleasant environment.

Prof. Dr. med. Giorgio Noseda
Bellinzona, June 2011

Foreword by Antonio Lanzavecchia
Director

This report witnesses the tenth anniversary of the Institute for Research in Biomedicine (IRB) that was celebrated with a series of scientific and public initiatives organized by our Director of Communication, Thomas Brooks, throughout 2010. On the scientific side, the IRB organized the 2010 USGEB meeting of the Swiss Biological Societies in Lugano, the annual congress of the Swiss Society for Allergology and Immunology and the 11th International Symposium on Dendritic Cells in Fundamental and Clinical Immunology: Forum of Vaccine Science. At this last meeting, that had more than 800 scientists in attendance, the International Society for Dendritic Cells and Vaccine Science was launched with the aim of fostering research in human immunology and to promote new approaches to vaccine discovery. Public initiatives included an IRB open day, exhibitions of photographs on poverty related diseases by Ivo Saglietti and the History of Immunology, as well as an event in the Teatro Sociale in Bellinzona.

The last two years witnessed an increased integration of the IRB into the Swiss academic environment. In 2009, the IRB became affiliated with the Università della Svizzera Italiana (USI) and I was appointed Professor of Human Immunology at the Swiss Federal Institute of Technology Zurich. Maurizio Molinari and Federica Sallusto became faculty members of the joint ETH/UNIZH graduate program, which further facilitates recruitment of PhD students.

The IRB continues to play a role in education by training PhD students enrolled in Swiss and Italian Universities. IRB students benefit from formal lectures by Group Leaders, regular journal clubs and a world-class PhD lecture course supported by the Gustav & Ruth Jacob Foundation. PhD students remain the driving force of the IRB and enjoy the open and friendly atmosphere of an English-speaking institute. At present, 25 graduate students work at the IRB and >40 have completed their training to date. Fresh doctorates, as well as senior postdocs, leaving the IRB have secured excellent positions in leading institutions in Europe, the USA and Asia.

Currently the IRB hosts eight research groups led by Fabio Grassi, Antonio Lanzavecchia, Maurizio Molinari, Silvia Monticelli, Federica Sallusto, Marcus Thelen, Mariagrazia Uguccioni and Luca Varani. The IRB is internationally recognized as a center of competence in human immunology. IRB researchers have pioneered studies on human dendritic cells, T cell activation, differentiation and migration, and have developed new methods for high throughput analysis of T and B cell repertoires. At the same time, research is performed in the mouse system when this is most appropriate or the only experimental system available. The research in the last two years has produced a variety of interesting results. Among those

are the role of ATP in balancing inflammatory and suppressor cells; the characterization of protective and pathogenic antibodies elicited by Dengue virus; the exploitation by Coronaviruses of a cellular quality control pathway to replicate the viral genome; the characterization of pan influenza neutralizing antibodies; and the identification of a novel subset of effector T cells dedicated to skin immunity.

IRB scientists have established an effective network of collaborations with leading institutions in Europe, America and Asia. The grants received from the Swiss National Science Foundation, the European Union, the European Research Council, the Bill & Melinda Gates Foundation, and the US National Institutes of Health also attest to their success. Our scientists are also supported by the Swiss Vaccine Research Institute (SVRI) and the Institute for Arthritis Research (IAR). The IRB recently received a generous support from a private foundation to recruit a Group Leader working on chronic inflammation and bone disease, improve the flow cytometry and imaging facilities, and develop an antigen production facility. The technologies to make human monoclonal antibodies have been validated in multiple projects on infectious and autoimmune diseases and were licensed by the IRB to a startup company, Humabs, that moved in January 2011 from the USA to Bellinzona, where it has established research laboratories. In this way the IRB has shown the capacity to act as an effective incubator for biotech companies.
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, June 2011

SECTION 1

RESEARCH PROJECTS

INDEX SECTION 1 – RESEARCH PROJECTS

Fabio Grassi
T Cell Development

Fabio Grassi

Fabio Grassi earned his degree in Medicine at the University of Pavia in 1985 and a Ph.D. in Microbiology at the University of Milan in 1993. He 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 communications 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

Group Leader: Fabio Grassi, MD, PhD > *fabio.grassi@irb.usi.ch*

Members: Michela Frascoli, *PhD student* – Jessica Marcandalli, *Diploma student* – Michele Proietti, *MD* – Tanja Rezzonico Jost, *PhD student* – Ursula Schenk, *PhD* – Giulia Siciliano, *PhD student*

Purinergic regulation of immunosuppressive regulatory T cells

Ursula Schenk, Michela Frascoli, Michele Proietti 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.

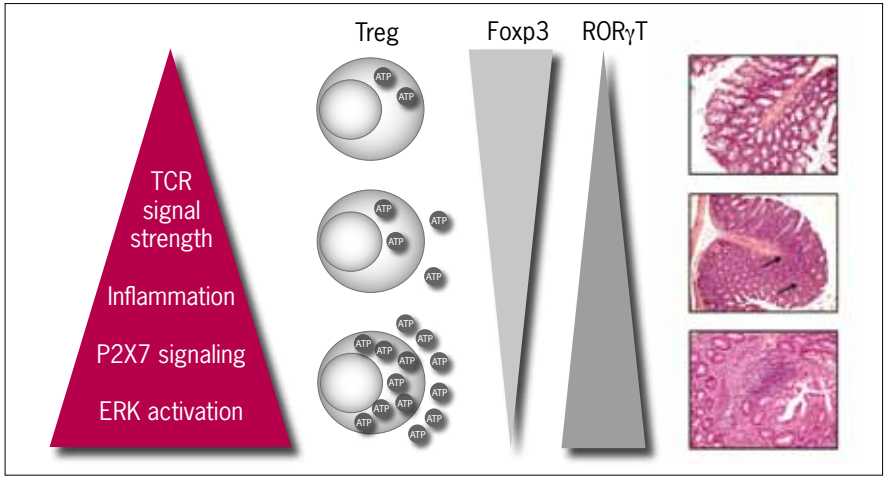
» ***Schenk U. et al.***
Sci Signal. 2011, 4:ra12.

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.



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 RORgt 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 p2rx7-/- 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.

Role of purinergic P2X7 receptor in T cell lineage choice

Michela Frascoli, Jessica Marcandalli 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 αβ 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

Michela Frascoli 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)

Giulia Siciliano 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 purinergic P2X receptors are important in regulating chemokine synthesis in the CNS and may be important in mediating communication with hematopoietic cells, we treated immuno-deficient mice, adoptively transferred with T-ALL cells, with a pharmacological antagonist of purinergic P2X receptors, periodate-oxidized 2,3-dialdehyde ATP (oATP). The aim of this project is to understand the molecular basis of the extended survival we observed in mice with T-ALL upon treatment with oATP.

B cell homeostasis in chronic inflammation

Anna Casati, Michela Frascoli and Fabio Grassi

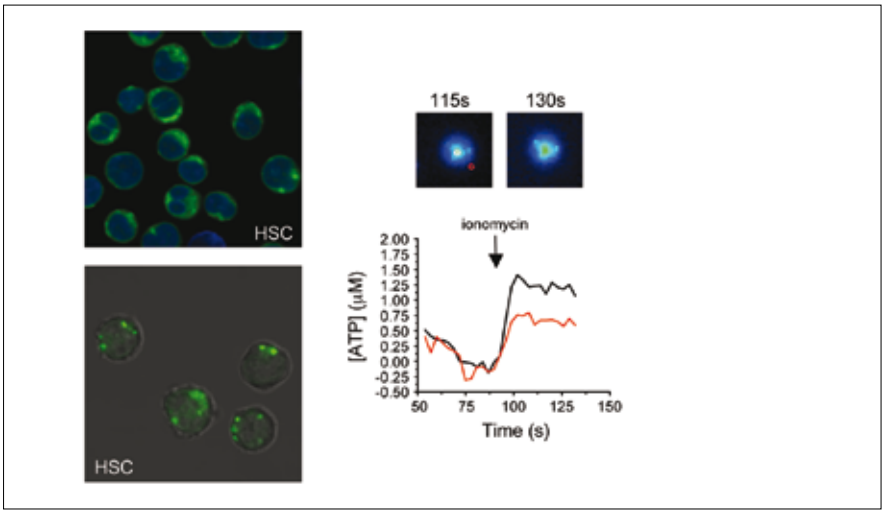
Inflammation promotes granulopoiesis over B lymphopoiesis in the bone marrow (BM). We studied B cell homeostasis in two murine models of T cell-mediated chronic inflammation, namely calreticulin-deficient fetal liver chimeras (FLC), which develop severe blepharitis and alopecia due to T cell hyper-responsiveness, and inflammatory bowel disease (IBD) caused by injection of naive $CD4^+$ T cells into lymphopenic mice. We have shown that despite the severe depletion of B cell progenitors during chronic, peripheral T cell-mediated inflammation, the population of BM mature re-circulating B cells was unaffected. These B cells are poised to differentiate to plasma cells in response to blood-borne pathogens, in an analogous fashion to non-re-circulating marginal zone (MZ) B cells in the spleen. MZ B cells nevertheless differentiated more efficiently into plasma cells upon polyclonal stimulation by Toll-like receptor (TLR) ligands, and were depleted during chronic T cell-mediated inflammation *in vivo*. The preservation of mature B cells in the BM was associated with an increased concentration of macrophage migration inhibitory factor (MIF) in the serum and BM plasma. MIF produced by perivascular dendritic cells (DC) in the BM provides a crucial survival signal for re-circulating B cells, and mice treated with a MIF inhibitor during inflammation showed significantly reduced mature B cells in the BM. These data indicate that MIF secretion by perivascular DC may promote the survival of the re-circulating B cell pool to ensure responsiveness to blood-borne microbes, despite loss of the MZ B cell pool that accompanies depressed lymphopoiesis during inflammation.

» ***Traggiai E. et al.*** *PLoS ONE 2010, 5:e11262.*

Development of an implantable system to monitor inflammation and metabolism

Tanja Rezzonico Jost, Michele Proietti, Giulia Siciliano 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 microelectronics 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)



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).

Funding

- *Swiss National Science Foundation*
  Purinergic signalling in T cell physiology
  310030_124745 / 2009-2012
- *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 Leonardo*
  Identification of purinergic antagonists for the treatment of autoimmune diseases
  2010
- *Fondazione per la ricerca sulla trasfusione e sui trapianti*
  Contribution of purinergic P2X7 receptor to T cell function
  2010
- *Fondazione Ticinese per la Ricerca sul Cancro*
  Purinergic signalling in the pathophysiology of central nervous system infiltration in T-cell leukaemia
  2010-2011

Collaborations

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Publications

- ***Hierarchy of immunosuppressive strength among myeloid-derived suppressor cell subsets is determined by GM-CSF.***
  Dolcetti, L., E. Peranzoni, S. Ugel, I. Marigo, A. Fernandez Gomez, C. Mesa, M. Geilich, G. Winkels, E. Traggiai, A. Casati, F. Grassi, and V. Bronte.
  Eur J Immunol. 2010; 40:22-35.

- ***Homeostatic expansion of autoreactive immunoglobulin-secreting cells in the Rag2 mouse model of Omenn syndrome.***
  Cassani, B., P. L. Poliani, V. Marrella, F. Schena, A.V. Sauer, M. Ravanini, D. Strina, C. E. Busse, S. Regenass, H. Wardemann, A. Martini, F. Facchetti, M. van der Burg, A. G. Rolink, P. Vezzoni, F. Grassi, E. Traggiai, and A. Villa.
  J Exp Med. 2010; 207:1525-1540.

- ***Selective preservation of bone marrow mature recirculating but not marginal zone B cells in murine models of chronic inflammation.***
  Traggiai, E., A. Casati, M. Frascoli, S. Porcellini, M. Ponzoni, F. Sanvito, L. Leng, R. Bucala, L. Moretta, and F. Grassi.
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- ***Purinergic control of neutrophil activation.***
  Grassi, F.
  J Mol Cell Biol. 2010; 2:176-177.

- ***Cell-autonomous regulation of hematopoietic stem cell cycling activity by ATP.***
  Casati, A., M. Frascoli, E. Traggiai, M. Proietti, U. Schenk, and F. Grassi.
  Cell Death Differ. 2011; 18:396-404.

- ***ATP inhibits the generation and function of regulatory T cells through the activation of purinergic P2X receptors.***
  Schenk U., M. Frascoli, M. Proietti, R. Geffers, E. Traggiai, J. Buer, C. Ricordi, A.M. Westendorf, and F. Grassi.
  Sci Signal. 2011; 4:ra12.

Lectures and Seminars

- ***Convegno "Una iniezione di speranza"***
  Attualità e Prospettive della Ricerca per il Diabete Mellito tipo 1
  Sistema immunitario e diabete
  Terni (IT) / 20.06.2009

- ***Translational Research in Paediatric Rheumatology***
  Adaptive Immunity and the Pathogenesis of Rheumatic Diseases
  Targeting T cells in autoimmune diseases
  Badia Benedettina della Castagna, Genoa (IT) / 24-27.09.2009

- ***SWISSIBDdays***
  Purinergic P2X receptor antagonism in the treatment of experimental inflammatory bowel disease
  Nottwil (CH) / 1-2.10.2009

- ***"A. Avogadro" University of Eastern Piedmont***
  Department of Medical Sciences
  Seminar"Signaling pathways in T cell homeostasis"
  Novara (IT) / 14.11.2009

- ***University Hospital Essen***
  Institute of Medical Microbiology,
  Seminar "Shaping T cell function by ATP"
  Essen (DE) / 24.11.2009

- ***San Raffaele Scientific Institute***
  Seminar"Shaping T cell function by ATP"
  Milan (IT) / 30.11.2009

- ***Istituto Clinico Humanitas***
  Lecture Course. Regulation of bone turnover: The Role of Cytokines, Hormones and Immune Cells
  Seminar"Inflammation and bone marrow"
  Rozzano (IT) / 26-27.04.2010

- ***Filarete Foundation***
  Seminar"Purinergic signalling in adaptive immunity"
  Milan (IT) / 14.05.2010

- ***Purines 2010***
  Tuning of T cell immunosuppressive function by ATP
  Tarragona-Barcelona (ES) / 30.5.2010-2.6.2010

- ***University of Basel***
  Department of Biomedicine
  Lecture Series in Advanced Immunology
  Seminar"Structural and functional features of the pre-TCR"
  Basel (CH) / 22.10.2010

- ***University of Basel***
  Department of Biomedicine
  Lecture Series in Advanced Immunology
  Seminar"ATP as a soluble mediator in adaptive immunity"
  Basel (CH) / 22.10.2010

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 three main themes. The first is the impact of innate immunity on the adaptive immune response, with special emphasis on the activation of dendritic cells and the production of polarizing cytokines. The second is the role of signal strength on T and B cell activation and the generation of effector and memory cells. The third is the development of high throughput culture methods to analyse the repertoires of human memory B cells and plasma cells and to isolate human monoclonal antibodies. We are currently applying these methods to study the organization and maintenance of human B cell memory, to investigate the mechanisms of protection versus pathology and to identify conserved epitopes in pathogens through the isolation of broadly neutralizing monoclonal antibodies. We are convinced that a systematic analysis of the human B and T cell response to human pathogens will have an impact on vaccine design and are therefore exploring an approach that we termed"analytic vaccinology".

Team

Group Leader: Antonio Lanzavecchia, MD > *lanzavecchia@irb.usi.ch*

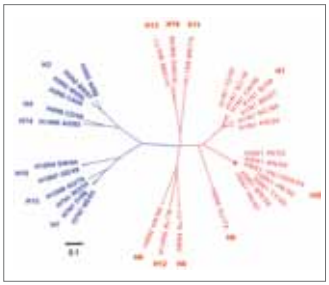
Members: Simone Becattini, *PhD Student* – Martina Beltramello, *PhD* – Davide Corti, *PhD* – Blanca Fernandez-Rodriguez, *Technician* – Anna Kabanova, *PhD* – Annalisa Macagno, *PhD* – Matteo Mauri, *PhD Student* – Andrea Minola, *Technician* – Leontios Pappas, *PhD Student* – Debora Pinna, *PhD* – Dora Pinto, *PhD Student* – Isabella Giacchetto, *Technician* – Chiara Silacci, *Technician* – Janine Stubbs, *PhD*

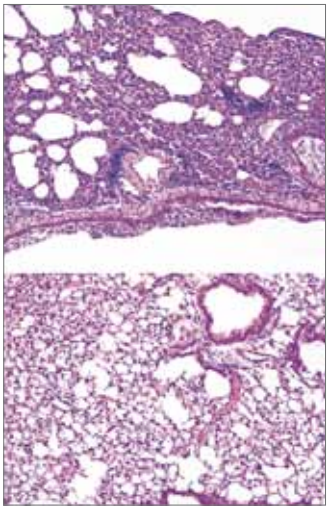
Heterosubtypic neutralizing antibodies produced following seasonal influenza vaccination

» *Corti D. et al.*
J Clin Invest. 2010, 120:1663-73.

Davide Corti, Debora Pinna, Chiara Silacci, Blanca M. Fernandez-Rodriguez, Fabrizia Vanzetta, Federica Sallusto and Antonio Lanzavecchia

The target of neutralizing antibodies that protect against influenza virus infection is the viral protein hemagglutinin (HA). Genetic and antigenic variation in HA has been used to classify influenza viruses into subtypes (H1–H16). The neutralizing antibody response to influenza virus is thought to be specific for a few antigenically related isolates within a given subtype. However, while heterosubtypic antibodies capable of neutralizing multiple influenza virus subtypes have been recently isolated from phage display libraries, it is not known whether such antibodies are produced in the course of an immune response to influenza virus infection or vaccine. We found that, following vaccination with seasonal influenza vaccine containing H1 and H3 influenza virus subtypes, some individuals produce antibodies that cross-react with H5 HA. By immortalizing IgG-expressing B cells from four individuals, we isolated 20 heterosubtypic mAbs that bound and neutralized viruses belonging to several HA subtypes (H1, H2, H5, H6, and H9), including the pandemic A/California/07/09 H1N1 isolate. The monoclonal antibodies (mAbs) used different VH genes and carried a high frequency of somatic mutations. With the exception of a mAb that bound to the HA globular head, all heterosubtypic mAbs bound to acid-sensitive epitopes in the HA stem region. Four mAbs were evaluated *in vivo* and protected mice from challenge with influenza viruses representative of different subtypes. These findings reveal that seasonal influenza vaccination can induce polyclonal heterosubtypic neutralizing antibodies that cross-react with the swine-origin pandemic H1N1 influenza virus and with the highly pathogenic H5N1 virus.
This work was done in collaboration with Kanta Subbarao, Laboratory of Infectious Diseases and NIAID, NIH, Rockville, MA, USA and Nigel Temperton and Robin Weiss, Centre for Medical Molecular Virology, University College London, London, UK.





↖ ***Phylogenetic tree of influenza A hemagglutinins***

← ***Sections of lungs of mice infected with the H1 pandemic influenza virus***
in the absence (upper panel) and in the presence (lower panel)of a broadly neutralizing antibody.

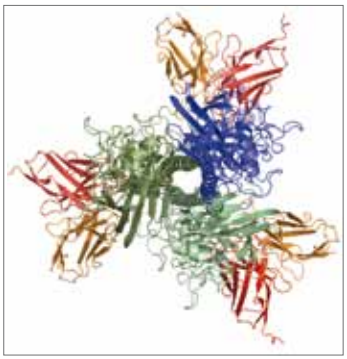
A pan influenza A neutralizing antibody selected from cultures of single human plasma cells

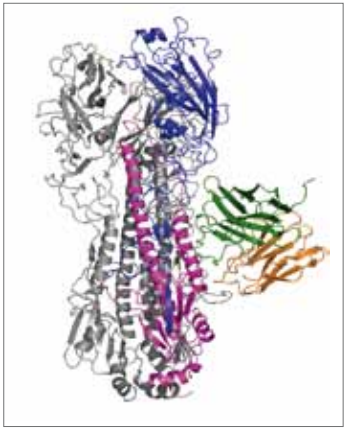
Davide Corti, Giosiana Codoni, Annalisa Macagno, David Jarrossay, Debora Pinna, Andrea Minola, Chiara Silacci, Blanca M. Fernandez-Rodriguez, Isabella Giacchetto-Sasselli, Federica Sallusto and Antonio Lanzavecchia

Using a novel single cell culture method that allows screening of large numbers of plasma cells, we repeatedly isolated from an immune donor a monoclonal antibody (FI6) that recognizes the HA glycoprotein and neutralizes Group 1 and Group 2 influenza A viruses. FI6 protected mice from lethal challenge with influenza virus both in prophylactic and thera-

peutic settings. 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. Attempts to select antibody-resistant escape mutants were unsuccessful. Complexes of the antibody with HAs from the Group 1 H1 (A/California/04/09) and the Group 2 H3 (A/Hong Kong/1/68) subtypes were analysed by X-ray crystallography. The structural data obtained indicate that, although the core epitope on helix A is similar to that recognized by the previously described Group 1-specific antibodies CR6261 and F10, FI6 binds with a different angle, 5-10 Å more membrane distal and contacts a larger area embracing helix A and extending to the fusion peptide of the neighbouring monomer both in the cleaved and un-cleaved forms. FI6 binding is mediated by both VH and VL CDRs, with a prominent contribution of the long HCDR3, which accommodates different conformations of the Group-specific Trp-21 loop, and of the heavily mutated LCDR1. The use of both VH and VL chains and the long HCDR3 are characteristic of naturally selected antibodies and contrast with the property of phage-derived antibodies CR6261 and F10 that bind using only VH residues. This is the first example of an HA-specific pan influenza A neutralizing antibody that can be used for passive protection and vaccine design.

This work was done in collaboration with John Skehel and Steve Gamblin, MRC, National Institute for Medical Research, The Ridgeway, Mill Hill, London, UK; Nigel Temperton, Viral Pseudotype Unit, School of Pharmacy, University of Kent, Kent, UK; Johannes Langedijk, Pepscan Therapeutics BV, Lelystad, NL; and Fabrizia Vanzetta and Gloria Agatic, Humabs BioMed, Bellinzona, CH.





↗ ***The crystal structure of FI6 Fab***
bound to influenza hemagglutinin trimer from the H1 swine pandemic virus

➜ ***The crystal structure of FI6 Fab***
bound to influenza hemagglutinin trimer from the H1 swine pandemic influenza virus

The effect of antibodies on the immune response to influenza virus

Leontios Pappas, Andrea Minola, Davide Corti and Antonio Lanzavecchia

We are interested to understand how passively administered antibodies affect the T and B cell response to vaccines or infectious agents as it occurs following serotherapy or following transfer of maternal antibodies in newborns. We are therefore characterizing the immune response of mice infected with influenza A virus PR8 in the absence or in the presence of passively administered monoclonal antibodies specific for different regions of the HA molecule. We are following the B cell response and the development of memory cells using a clonal assay to dissect the fine specificity and crossreactivity of the antibodies produced. Preliminary results indicate that passively administered neutralizing antibody can protect from clinical pathology without compromising the total neutralizing antibody response to the virus. We will also establish whether the passively administered antibody can modulate the T cell response, for instance by targeting antigen to dendritic cells and modifying its processing, and whether this effect may in turn influence the ability of those cells to interact

with antigen specific B cells. We are also attempting to identify priming conditions or modified antigens that would elicit heterosubtypic antibody responses. To achieve this ambitious goal we are considering both prime boost immunization as well as the design of modified HA molecules.

Novel monoclonal antibodies with neutralizing breadth from HIV-1-infected individuals

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

The isolation of human monoclonal antibodies (mAbs) that neutralize a broad spectrum of primary HIV-1 isolates and the characterization of the human neutralizing antibody B cell response to HIV-1 infection are important goals that are central to the design of an effective antibody-based vaccine. We immortalized IgG^+ memory B cells from individuals infected with diverse clades of HIV-1 and selected on the basis of plasma neutralization profiles that were cross-clade and relatively potent. Culture supernatants were screened using various recombinant forms of the envelope glycoproteins (Env) in multiple parallel assays. We isolated 58 mAbs that were mapped to different Env surfaces, most of which showed neutralizing activity. One mAb in particular (HJ16) specific for a novel epitope proximal to the CD4 binding site on gp120, selectively neutralized a multi-clade panel of Tier-2 HIV-1 pseudoviruses, and demonstrated reactivity that was comparable in breadth, but distinct in neutralization specificity, to that of the other CD4 binding site-specific neutralizing mAb b12. HJ16 was used to define a conserved region of the CD4 binding site, which has been defined as "core epitope" formed by an immunodominant triad of conserved amino acids (D474, M475, R476) at the outer domain/inner domain junction of gp120 that could not be mutated without loss of viral fitness. 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. HGN194 was able to provide sterilizing immunity to macaques challenged mucosally with a clade C SHIV isolate while inducing gag-specific $CD4^+$ and $CD8^+$ T cells. A third mAb (HK20) with broad neutralizing activity, particularly as a Fab fragment, recognized a highly conserved epitope in the HR-1 region of gp41, but showed striking assay-dependent selectivity in its activity. This study reveals that by using appropriate screening methods, a large proportion of memory B cells can be isolated that produce mAbs with HIV-1 neutralizing activity. Three of these mAbs show unusual breadth of neutralization and therefore add to the current panel of HIV-1 neutralizing antibodies with potential for passive protection and template-based vaccine design.

This work was done in collaboration with the Bill and Melinda Gates Foundation VDC consortium led by Robin Weiss, University College London, London, UK and Quentin Sattenteau, Dunn School of Pathology, University of Oxford, Oxford, UK; Fabrizia Vanzetta and Gloria Agatic, Humabs BioMed, Bellinzona, CH; Michel Nussenzweig, Rockefeller University, New York, NY, USA; and Ruth Ruprecht, Dana Farber, Boston, MA, USA.

» ***Corti D. et al.*** *PLoS ONE 2010, 5:e8805.*

» ***Pietzsch J. et al.*** *J Exp Med. 2010, 207:1995-2002.*

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

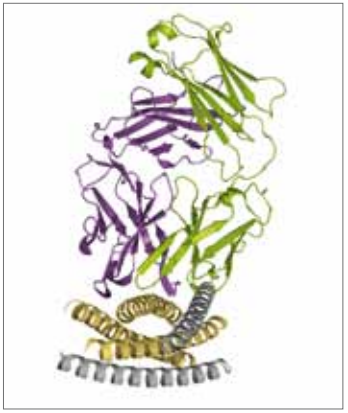
Characterization of mAb HK20 targeting the HR1 of HIV-1 gp41

Davide Corti, Chiara Silacci, Federica Sallusto and Antonio Lanzavecchia

The human monoclonal antibody (mAb) HK20 neutralizes a broad spectrum of primary HIV-1 isolates by targeting the highly conserved heptad repeat 1 (HR1) of gp41, which is transiently exposed during HIV-1 entry. Here we present the crystal structure of the HK20 Fab in complex with a gp41 mimetic 5-Helix at 2.3 Å resolution. HK20 employs its heavy chain CDR H2 and H3 loops to bind into a conserved hydrophobic HR1 pocket that is occupied by HR2 residues in the gp41 post fusion conformation. Compared to the previously described HR1-specific mAb D5, HK20 approaches its epitope with a different angle, which might favor epitope access and thus contribute to its higher neutralization breadth and potency. Comparison of the neutralization activities of HK20 IgG, Fab and scFv employing both single cycle and multiple cycle neutralization assays revealed much higher potencies for the smaller Fab and scFv over IgG, implying that the target site is difficult to access for complete antibodies. Nevertheless, two thirds of sera from HIV-1 infected individuals contain significant titers of HK20-inhibiting antibodies. The breadth of neutralization of primary isolates across all clades, the higher potencies for C-clade viruses and the targeting of a distinct site as compared to the fusion inhibitor T-20 demonstrate the potential of HK20 scFv as a therapeutic tool.

This work was done in collaboration with Winfried Weissenhorn, Unit of Virus Host Cell Interactions, UMI 3265, Universite Joseph Fourier-EMBL-CNRS, Grenoble, FR in the framework of the VDC consortium and with Fabrizia Vanzetta and Gloria Agatic, Humabs BioMed, Bellinzona, CH.

» ***Sabin C. et al.***
PLoS Pathog 2010, 6:e1001195.



➜ ***The HK20 Fab bound to the heptad repeat 1 of HIV gp41***

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

Janine Stubbs and Antonio Lanzavecchia

It is widely accepted that antibody responses against the human parasitic pathogen Plasmodium falciparum protect the host from the rigors of 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 and polyclonal antibodies in eliciting phagocytosis and parasite growth inhibition via Fcg 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 Fcg 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 im-

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

» *Barfod L. et al.* *J Immunol. 2010, 185:7553-61.*

munity 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, we are closing this programme due to lack of suitable samples.

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

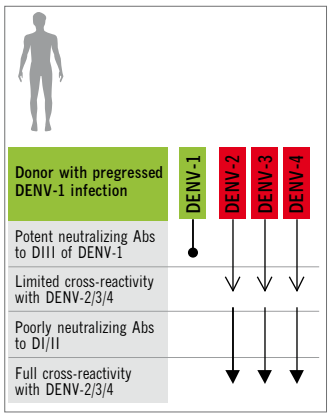
The human immune response to Dengue virus is dominated by highly cross-reactive antibodies endowed with neutralizing and enhancing activity

Martina Beltramello, Annalisa Macagno, Antonio Lanzavecchia and Federica Sallusto

» *Beltramello M. et al.* *Cell Host Microbe 2010, 8:271-83.*

Antibodies protect against homologous Dengue virus (DENV) infection but can precipitate severe dengue by promoting heterotypic virus entry via Fcg receptors (FcγR). We immortalized memory B cells from individuals after primary or secondary infection and analysed anti-DENV monoclonal antibodies (mAbs) thus generated. mAbs to envelope (E) protein domain III (DIII) were either serotype specific or cross-reactive and potently neutralized DENV infection. DI/DII- or viral membrane protein prM-reactive mAbs neutralized poorly and showed broad cross-reactivity with the four DENV serotypes. All mAbs enhanced infection at subneutralizing 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. Our findings reveal an unexpected degree of cross-reactivity in human antibodies against DENV and illustrate the potential for an antibody-based therapy to control severe dengue.

This work was done in collaboration with Luca Simonelli and Luca Varani, IRB, Bellinzona; Felix Rey, Institute Pasteur, Paris, FR; Mike Diamond, Washington University School of Medicine, St. Louis, MO, USA; and Eva Harris, University of California, Berkeley, Berkeley, CA, USA.



← ***The role of domain-specific antibodies in dengue protection or immunopathology.***

Intracellular immunity against non-enveloped viruses

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

Humoral immunity to enveloped viruses is mediated by different mechanisms such as inhibition of virus binding to target cells, inhibition of membrane fusion or recruitment of complement or FcR^+ killer cells against virus infected cells. Recent studies indicate that in the case of non-enveloped viruses, IgG antibodies can also mediate intracellular immunity by promoting viral degradation through recruitment of the cytosolic FcR TRIM21 (Mallery et

al. PNAS, 2010). Noroviruses are non-enveloped viruses that are responsible for most cases of viral gastroenteritis and undergo a continuous antigenic drift. From immune donors, we isolated a panel of monoclonal antibodies that are either specific for a particular genogroup or broadly crossreactive, including an antibody that binds to an epitope conserved in norovirus isolates from 1974 to 2010 and that overlaps with the binding site for the cellular receptor. Other examples are polyoma viruses such as JCV or BKV that are widespread in the population and cause severe disease in immunosuppressed individuals. By screening large numbers of healthy blood donors, we identified individuals with high titers of serum antibodies against JCV or BKV and isolated several monoclonal antibodies. Using fluorescent reporter viruses, we will investigate the relative role played by extracellular versus intracellular immunity.

This work is done in collaboration with Ralph Baric, University of North Carolina Chapel Hill, USA and Hans Hirsch, University of Basel, CH.

Human monoclonal antibodies that potently neutralize HCMV by targeting the gH/gL/UL128-131A complex

Annalisa Macagno, Nadia Bernasconi, Federica Sallusto and Antonio Lanzavecchia

Human cytomegalovirus (HCMV) is a widely circulating pathogen that causes severe disease in immunocompromised patients and infected foetuses. By immortalizing memory B cells from HCMV-immune donors, we isolated a panel of human monoclonal antibodies that neutralized at extremely low concentrations (IC90 values ranging from 5 to 200 pM) HCMV infection of endothelial, epithelial and myeloid cells. With the single exception of an antibody that bound to a conserved epitope in the UL128 gene product, all other antibodies bound to conformational epitopes that required expression of two or more proteins of the gH/gL/UL128-131A complex. Antibodies against gB, gH or gM/gN were also isolated and, albeit less potent, were able to neutralize infection of both endothelial-epithelial cells and fibroblasts. A soluble gH/gL/UL128-131A pentameric complex was produced by co-transfection using conventional plasmids or adenoviral vectors 5 genes including a gH with a deletion in the transmembrane region. The secreted complexes expressed all the epitopes recognized by conformation-dependent antibodies and could be used to detect antibodies in the serum of infected individuals. This study describes unusually potent neutralizing antibodies against HCMV that might be used for passive immunotherapy and identifies, through the use of such antibodies, novel antigenic targets in HCMV for the design of immunogens capable of eliciting previously unknown neutralizing antibody responses.

This work was done in collaboration with Fabrizia Vanzetta and Gloria Agatic, Humabs BioMed, Bellinzona, CH and Giuseppe Gerna, Fondazione IRCCS Policlinico San Matteo, Pavia, IT.

» ***Macagno A. et al.*** *J Virol. 2010, 84:1005-13.*

HCMV glycoprotein complexes in viral tropism and function

Anna Kabanova and Antonio Lanzavecchia

To understand the cellular tropism of HCMV, identify the molecular targets and better characterize the protective T and B cell response, we produced viral glycoprotein complexes in a soluble form. The gH/gL/UL128-131A complex, the gH/gL/gO complex and gB were produced in transfected cells, purified and used to identify the target molecules on epithelial cell and fibroblasts using co-precipitation and mass spectrometry (MS) analysis. Glycoprotein-specific and site-specific serological assays have been 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. Finally, to explore the possibility of developing a subunit vaccine we immunized mice with various glycoprotein complexes using different adjuvants and measured the neutralizing antibody response.

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

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, Milano, IT.

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

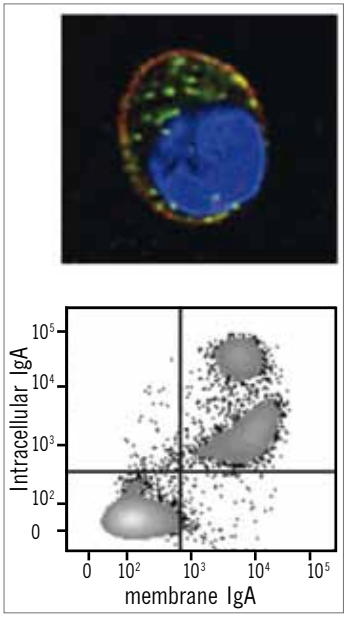
Dora Pinto and Antonio Lanzavecchia

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 plasma cells

Dora Pinto, Antonio Lanzavecchia and David Jarrossay

Plasma cells are terminally differentiated cells of the B cell lineage that secrete IgM, IgG or IgA antibodies at a high rate and are thought to lack the expression of membrane bound Ig. Long-term maintenance of plasma cells in the bone marrow is dependent on signals from chemokines, cytokines and adhesion molecules present in survival niches, while IgG immune complexes have been shown to increase plasma cell turnover by triggering FcγRIIB/CD32-mediated apoptosis. We found that human IgA and IgM plasma cells from the bone marrow or lamina propria, unlike IgG plasma cells, do not express CD32 and are not susceptible to killing by IgG immune complexes. Instead, they express membrane IgA or IgM that determine death or survival depending on the strength of Ig crosslinking. Signalling from membrane IgA or IgM occurs in the absence of the Igα/Igβ heterodimer and leads to ERK1/2 phosphorylation, Ca^{++} flux and upregulation of the survival receptor CD44. These findings demonstrate fundamentally distinct mechanisms for the homeostasis of IgG and IgA plasma cells and regulation of antibody levels.



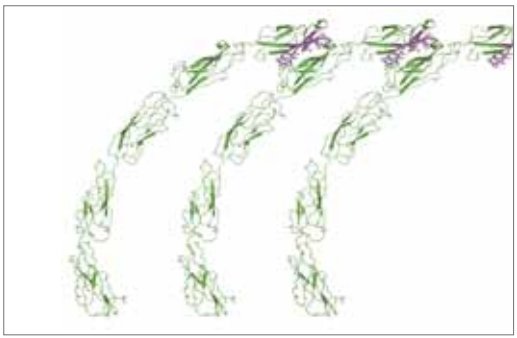
➜ ***Expression of the BCR on IgA plasma cells.***

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

Giulia Di Lullo, Davide Corti and Antonio Lanzavecchia

Pemphigus vulgaris (PV) is a life-threatening autoimmune blistering disease of skin and mucous membranes associated with autoantibodies against the cadherin-type adhesion molecules desmoglein (Dsg)3 and Dsg1 leading to intraepithelial blister formation. At present the involvement of anti-Dsg antibodies in PV pathogenesis is well established, while the mechanism of blister formation is only partly defined. We immortalized IgG^+ B cells from PV patients and isolated a panel of monoclonal antibodies specific for Dsg1 and Dsg3 that were characterized for their domain specificity and for their capacity to disaggregate keratinocytes *in vitro* and *in vivo* in a mouse model. Only a fraction of the Dsg3 antibodies were pathogenic and interestingly, one of them (PFA224) was found to bind the EC1 domain on the site that is expected to interact in cis with the EC2 of the neighboring molecule. This finding suggests that human pathogenic antibodies can act in cis by disrupting the array of Dsg3 molecules. Removal of somatic mutations from PFA224 abolished binding to Dsg3 indicating that this B cell clone was not initially autoreactive but acquired autoreactivity in the course of an immune response driven by a different, possibly foreign, antigen. We are characterizing the remaining antibodies to determine their fine specificity and the role of somatic mutations.

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.

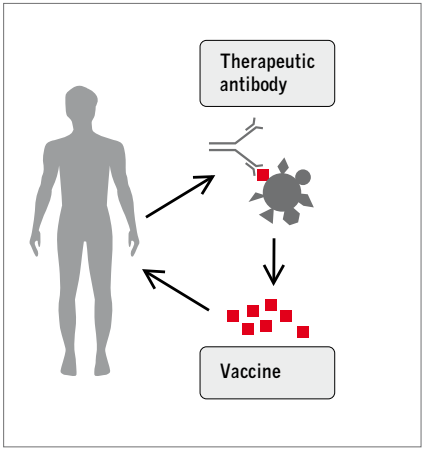


Cis interaction of desmogleins disrupted by autoantibodies.

Analytic Vaccinology

Antonio Lanzavecchia and Federica Sallusto

This approach is based on the high throughput analysis of the human immune response to pathogens or vaccines in order to identify the specificity and class of protective immunity. This information is then used to design antigens and adjuvants capable of eliciting a protective response of the same specificity and class. For B cells, we start from the analysis of the human antibody response to isolate human 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. For T cells, on the one hand we analyse pathogen specific memory T cells to identify the antigens and epitopes recognized as well as the class of response, as defined by the expression of cytokines and homing receptors. On the other hand, we reconstruct human T cell priming *in vitro* in order to dissect the mechanisms of T cell polarization and eventually identify the most appropriate adjuvants. Examples of these approaches are found in recent publications and reviews from our group.



The use of human monoclonal antibodies against infectious agents

» ***Sallusto F. et al.*** *Immunity 2010, 33:451-63.*

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

» ***Lanzavecchia A. et al.*** *Curr Opin Immunol. 2009, 21:298-304.*

» ***Ertl H.C. et al.*** *Curr Opin Immunol. 2010, 22:355-7.*

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- ***Bill & Melinda Gates Foundation***
  VDAC: Vaccine-induced protective cross-neutralization of HIV-1
  36637/ 2006-2011
- ***Bill & Melinda Gates Foundation***
  PTVDC: Poxvirus T cell vaccine discovery consortium
  38599/ 2006-2011
- ***CARIPLO Foundation***
  Development of a human cytomegalovirus vaccine based on glycoprotein complex including the UL128-131A gene products / 2010-2013
- ***CARIPLO Foundation***
  Novel strategies of vaccine design to prevent emerging and pandemic influenza virus infections (NoFlu) / 2010-2013
- ***CARIPLO Foundation***
  Human monoclonal antibodies as vaccine adjuvants and TLR agonist combinations as vaccine adjuvants / 2010-2013
- ***European Union***
  IMECS: Identification of mechanisms correlating with susceptibility for avian influenza
  FP7-HEALTH-F3-2008-201169 / 2008-2012
- ***European Union***
  PEMPHIGUS: Pemphigus: from autoimmunity to disease
  FP7-HEALTH-2007-200515 / 2008-2011
- ***European Union***
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- *Giuseppe Gerna*
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Lectures and Seminars

- ***Sanquin Research***
  Serological memory
  Amsterdam (NL) / 11.05.2009

- ***10th International Congress of Dermatology***
  Prague (CZ) / 21.05.2009

- *2009 Onassis Lecture Series*
  T lymphocyte differentiation, migration and immune regulation
  Dissecting the human antibody response to pathogens
  Heraklion, Crete (GR) / 29.07.2009
- *Miltenyi Biotec 20th Anniversary Symposium*
  Bergisch Gladbach (DE) / 14.08.2009
- *2nd European Congress of Immunology*
  Berlin (DE) / 15.09.2009 and 16.09.2009
- *SGKC/SSCC Congress*
  Lugano (CH) / 17.09.2009
- *Aging and Immunity*
  Siena (IT) / 21.09.2009
  6° Carlo Urbani Memorial Symposium
  Milano (IT) / 02.10.2009
- *Translational medicine in Multiple Myeloma*
  Milano (IT) / 09.10.2009
- *SIICA Workshop*
  Siena (IT) / 17.10.2009
- *Berlin Life Science Colloquium*
  Berlin (DE) / 22.10.2009
- *Lecture Series in Advanced Immunology*
  Basel (CH) / 20.11.2009
- *FLUSECURE Meeting*
  Bilthoven (NL) / 14.01.2010
- *Humanitas Lecture*
  On the cellular basis of immunological memory
  Milan (IT) / 23.02.2010
- *Institute Pasteur Vaccinology Course 2010*
  Paris (FR) / 03.03.2010
- *World Immune Regulation Meeting IV*
  Davos (CH) / 01.04.2010
- *4th European Congress of Virology*
  Cernobbio (IT) / 08.04.2010
- *The 15th International Conference on Human Antibodies & Hybridomas*
  Porto (PT) / 14.04.2010
- *Human Immune Response Workshop*
  Basel (CH) / 04.05.2010
- *Abbott sponsored symposium on Infection and Immunity*
  Chicago (US) / 14.05.2010
- *Immunochemistry & Immunobiology Gordon Conference*
  Les Diablerets (CH) / 20.05.2010
- *Novo Nordisk Seminar*
  Copenhagen (DK) / 25.05.2010
- *BioBusiness Advanced short course on BioEntrepreneurship*
  Lugano (CH) / 07.06.2010
- *D-BIOL Symposium*
  Zurich (CH) / 09.06.2010
- *4th MASIR European Conference*
  Mykonos (GR) / 11.09.2010
- *Lugano Stem Cell Meeting 2010*
  Lugano (CH) / 22.06.2010
- *China Tregs/Th17 2010*
  Shanghai (CNY) / 19.07.2010
- *The 14th International Congress of Immunology*
  Kobe (JP) / 24.08.2010
- *From the Laboratory to the Clinic*
  Oxford (UK) / 21.09.2010
- *7th GARN Meeting*
  Zurich (CH) / 08.10.2010
- *PostFinance Forum*
  Bellinzona (CH) / 15.10.2010
- *Institute for Medical Microbiology*
  Seminar"Dissecting the human immune response to pathogens"
  Basel (CH) / 21.10.2010
- *Pfizer Bio Therapeutics*
  Cambridge (US) / 26.10.2010
- *Keystone Symposia on Molecular and Cellular Biology*
  Seattle (US) / 28.10.2010
- *The French Society for Immunology Annual Meeting*
  Marseille (FR) / 25.11.2010

- *ConfrontTi10*
  Ascona (CH) / 30.11.2010
- *The Swiss Society of Nephrology Annual Meeting*
  Lugano (CH) / 03.12.2010
- *CMCBI Educational Day and Seminar Series*
  Dissecting the human memory B cell and plasma call response to pathogens and vaccines
  London (UK) / 24.02.2011
- *Utrecht University Eijkman Seminar Series*
  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*
  Paris (FR) / 14.03.2011

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) is the site of maturation for proteins destined to the extracellular space, the plasma membrane and to the organelles of the endocytic and secretory pathways. The ER contains molecular chaperones and folding enzymes that assist to attain the native, functional conformation of newly synthesized polypeptides. Mutations, deletions and truncations of the polypeptide chains may cause protein-misfolding diseases characterized by a"loss-of-function"when the mutant protein is degraded or by a"gain-of-toxic-function"when the aberrant polypeptide undergoes aggregation.

The aim of our work is to understand the molecular mechanisms regulating chaperone-assisted protein folding and protein disposal from the mammalian ER. We are also interested in understanding the regulation of chaperone content in the ER lumen. A thorough knowledge of these processes is essential in learning how to intervene in protein biogenesis and how to manipulate protein folding, quality control and degradation to delay progression or even to cure diseases caused by inefficient functioning of the cellular protein factory. It will also increase the efficiency of protocols for ectopic expression of recombinant proteins to be employed in the clinics and in industry.

Team

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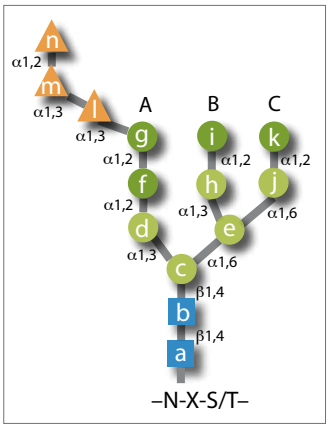
Characterization of Malectin, a Novel ER-Resident Lectin

Carmela Galli and Maurizio Molinari

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

In the ER lumen, asparagine residues in nascent polypeptide chains are modified with a pre-assembled $Glc_3Man_9GlcNAc_2$- oligosaccharide (Figure 1). 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 Metazoa 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. Studies to characterize binding specificity of Malectin in the living cell are ongoing.



↖ *Figure 1*

The core oligosaccharide added onto side chains of asparagine residues in a specific sequon (N=asparagine, X=any amino acid except proline, S/T=serine or threonine) comprises three glucose (triangles), nine mannose (circles) and two N-acetylglucosamine (squares) residues.

Substrate-Specific Mechanisms of Protein Degradation from the ER

Riccardo Bernasconi and Maurizio Molinari

» *Bernasconi R. et al.* *J Cell Biol. 2010, 188:223-235. - Highlights J Cell Biol. 2010, 188:176.*

Sophisticated quality control mechanisms prolong retention of protein folding intermediates in the ER until maturation, while sorting out terminally misfolded polypeptides for ER-associated degradation (ERAD). The presence of structural lesions in the luminal, transmembrane or cytosolic domains determines the classification of misfolded polypeptides as ERAD-L, ERAD-M or ERAD-C substrates and results in selection of distinct degradation

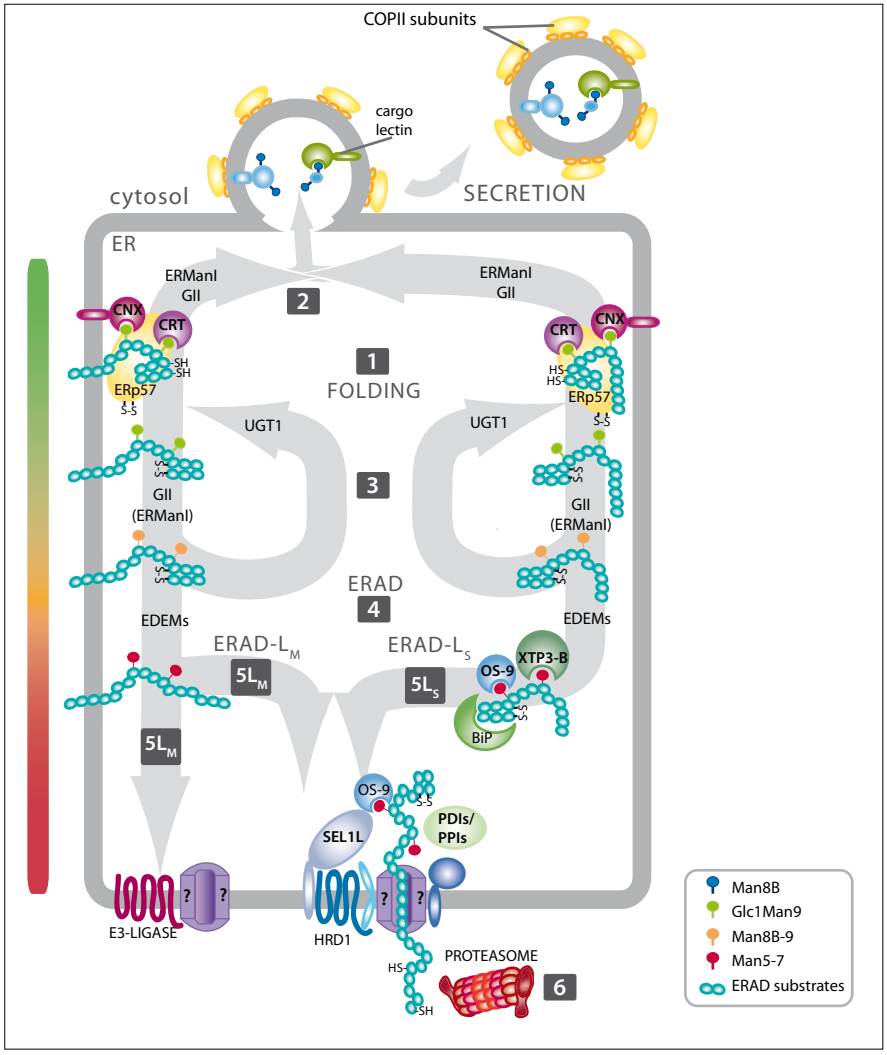


Figure 2

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 glucose residue on the mannose residue g (step 3). Extensive de-mannosylation irreversibly extracts terminally misfolded polypeptides from the CNX cycle (step 4). ERAD-L$_S$ and ERAD-L$_M$ 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).

pathways (Figure 2). Our studies demonstrated that disposal of Soluble (non-transmembrane) polypeptides with luminal lesions (ERAD-L_S 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). Our data revealed that, in contrast to budding yeast, tethering of mammalian ERAD-L substrates to the membrane changes selection of the degradation pathway.

Cyclophilins Intervention in Protein Biogenesis and Quality Control in the Mammalian ER

Riccardo Bernasconi, Tatiana Soldà, Carmela Galli and Maurizio Molinari

The oxidoreductase-catalyzed formation of intra- and inter-molecular disulfide bonds and the peptidyl-prolyl cis/trans isomerase (PPI)-catalyzed cis/trans isomerization of peptidyl-prolyl bonds are rate-limiting steps for folding of nascent polypeptides. A role of oxidoreductases in polypeptide maturation is well documented. On the other hand, even though the involvement of PPI family members in protein refolding has been established in test tube experiments, no data is available on the involvement of this class of enzymes in protein folding, quality control or disposal in the ER of living cells. In our studies, we have shown that the immunosuppressive drug CsA, a specific inhibitor of the cyclophilin family of PPIs, selectively delays the degradation of the ERAD-L_S substrate BACE457Δ leaving unaffected disposal from the ER of the same polypeptide when tethered to the ER membrane (the ERAD-L_M protein BACE457). This identifies CsA as the first inhibitor that selectively acts upon an ERAD-L_S substrate and not upon the corresponding ERAD-L_M polypeptide (Figure 2). We have extended this finding by showing that, among roughly 20 mammalian cyclophilin family members, CyPB is unique because it plays a crucial role in ERAD. CyPB function in the disposal of misfolded polypeptides from the mammalian ER requires its enzymatic activity. Importantly, CsA is not a general inhibitor of the ERAD-L_S pathway and CyPB is not required for disposal of all ERAD-L_S substrates. Rather, the presence of peptidyl-prolyl bonds in the cis conformation renders disposal of ERAD-L_S substrates sensitive to CsA and dependent on CyPB intervention. Altogether, our work presents the first evidence for the enzymatic involvement of a PPI in protein quality control in the ER of a living cell.

» *Bernasconi R. et al. PLoS ONE 2010, 5:e13008.*

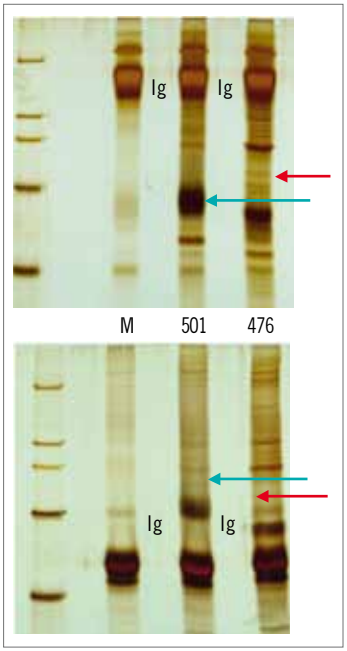
Identification of Novel ER-Resident Quality Control Players by "Interactomics"

Jessica Merulla, Tatiana Soldà, Carmela Galli 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 will prepare a series of stable human cell lines expressing model, epitope-tagged, folding-competent and folding-defective

proteins. The model proteins are used as baits to capture interacting partners in the same immuno-complexes. Preliminary experiments (Figure 3) 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. The proteins co-immuno-isolated with the individual baits have been subjected to tryptic digestion and fragments have been separated by nano-HPLC followed by tandem MS. Fragmentation spectra of the samples have been 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.

We will also determine how variations in bio-physical features of the model proteins (e.g. protein topology, glycosylation state, oxidation state, conformation of peptidyl-prolyl bonds) affect the molecular composition of the model-protein's interactome.



➜ ***Figure 3***

Isolation of immuno-complexes containing the folding-competent BACE501 (green arrowheads) or the folding-defective BACE476 (red arrowheads). M is the immuno-isolate of Mock-transfected HEK293 cells, 501 and 476 are the immuno-isolates of HEK293 cells stably expressing BACE501 and BACE476, respectively. Ig is the αBACE immunoglobulin chain used for the immunoprecipitation. Co-precipitating polypeptides are identified as described in the text.

Disposal of Non-Glycosylated Polypeptides from the Mammalian ER

Jessica Merulla, Tatiana Soldà, Carmela Galli 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 folding ER environment to eventually attain the native architecture 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, versus extraction from, the folding environment. In particular, the slow and progressive removal of terminal α1,2-bonded mannose residues (Figure 1) from protein-bound oligosaccharides irreversibly tags newly synthesized polypeptides for degradation. Virtually nothing is known about quality control of non-glycosylated polypeptides. To study this issue, we will generate 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.

E3 Ubiquitin Ligases Regulating Disposal of Membrane-Tethered Misfolded Polypeptides

Tatiana Soldà and Maurizio Molinari

The capacity to rapidly remove folding-defective polypeptides from the ER lumen is crucial to maintain cell homeostasis. Several ER-resident proteins survey the maturation of incoming cargo to eventually interrupt unsuccessful folding-attempts and to facilitate dislocation of terminally misfolded polypeptides across the ER membrane for degradation by cytosolic proteasomes. Current models claim that dislocation of misfolded proteins across the ER membrane is regulated by multi-protein complexes built around membrane-embedded E3 ubiquitin ligases that facilitate the proteasome-dependent degradation of proteins extracted from the ER lumen by polyubiquitylating the misfolded chains. Studies performed in our lab (Bernasconi et al. 2010) led to establish a crucial involvement of HRD1 in disposal of soluble polypeptides (ERAD-L_S substrates) from the ER lumen. HRD1 was found to be dispensable for disposal of polypeptides with the same folding defect, when these where anchored to the ER membrane (ERAD-L_M substrates) (Figure 2). The aim of this project is to understand which one, of the several E3 ligases in the mammalian ER membrane (e.g. Synoviolin/HRD1, gp78, TEB4, Trc8, RFP2, RMA1) and in the cytosol (e.g. CHIP, Parkin, Fbx2) is involved in disposal of ERAD-L_M substrates.

The Selective Clearance of ERAD Regulators from the ER Lumen

Riccardo Bernasconi, Julia Noack, Carmela Galli and Maurizio Molinari

EDEM1 is a crucial regulator of ERAD that extracts non-native glycopolypeptides from the CNX chaperone system (Figure 2). Under normal growth conditions, the intralumenal level of EDEM1 must be low to prevent premature interruption of ongoing folding programs. Our studies showed that in unstressed cells, EDEM1 is segregated from conventional ER-resident chaperones, is released from the ER in LC3-I-coated vesicles and is rapidly degraded. The rapid turnover of EDEM1 is regulated by a poorly characterized mechanism that shows similarities, but is clearly distinct, from macroautophagy. We have defined as ERAD tuning the non-transcriptional mechanisms operating in the mammalian ER at steady state to rapidly remove EDEM1 (and other ERAD regulators) from the ER lumen (Figure 4). We propose that ERAD tuning is crucial to maintain the capacity of cells to efficiently produce polypeptides at steady state. In fact, cells with defective ERAD tuning contain unphysiologically high levels of several ERAD regulators, show enhanced ERAD activity and are crucially

characterized by impaired capacity to efficiently complete maturation of model glycopolypeptides. The aim of this project is the identification of the proteins whose intralumenal level is regulated by ERAD tuning, the mechanisms of segregation of these proteins from the long-lived chaperones that are retained in the bulk ER, and the mechanisms regulating the vesicular transport out of the ER. Since the vesicular transport out of the ER of select ERAD factors 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.

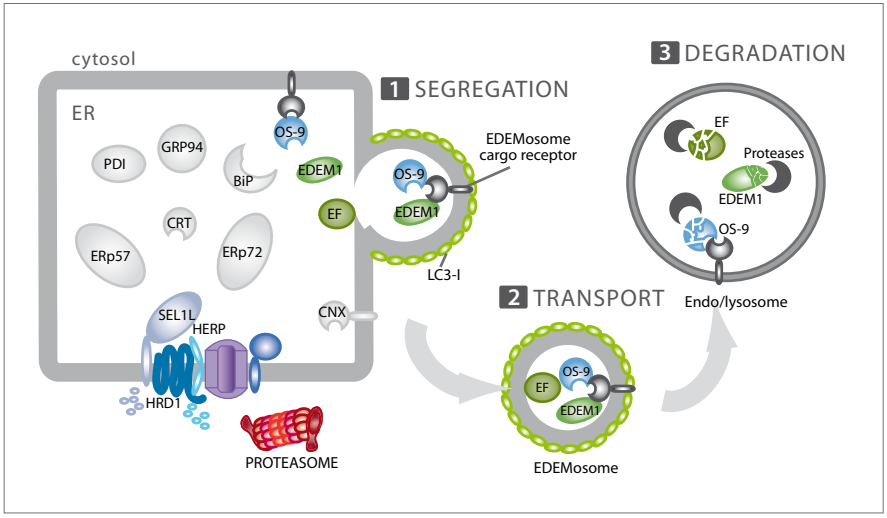


Figure 4

Many ERAD regulators are short-lived proteins at steady state. Some of them are degraded with the intervention of cytosolic proteasomes (e.g. SEL1L and HERP). The selective removal of EDEM1 and OS-9 from the ER can be subdivided in three steps. 1) Association with an elusive receptor (identified in our studies as SEL1L) allows segregation of EDEM1, OS-9 and possibly other ERAD factors (EF) from conventional, long-lived ER-resident chaperones (in grey); 2) The ERAD regulators exit the ER in small, LC3-I-coated vesicles, the EDEMosomes; 3) EDEMosomes deliver their content to endo/lysosomal compartments for disposal.

ER-Derived Vesicles Involved in ERAD Tuning Are Hijacked for Coronavirus Replication

Riccardo Bernasconi, Siro Bianchi and Maurizio Molinari

» ***Reggiori F. et al.*** *Cell Host & Microbe 2010, 7:500-508.*
• *Highlights, Cell Host & Microbe 2010, 7:424-426.*
• *Editors' Choice, Science 329, 14.*
• *Leading Edge Microbiology Select, Cell 142, 5.*
• *Recommended by the Faculty of 1000.*

In collaboration with Fulvio Reggiori (University Medical Centre Utrecht) and Cornelis A.M. de Haan (Utrecht University), we have established that Coronaviruses (CoV) co-opt the host cell machinery by regulating the vesicular export of rapidly turned-over ERAD factors to derive cellular membranes for replication. Mouse hepatitis virus (MHV) infection causes accumulation of EDEM1, and OS-9, another short-lived ER chaperone, in double membrane vesicles coated with the non-lipidated LC3/Atg8 autophagy marker (Figure 5). Downregulation of LC3, but not inactivation of host cell autophagy, protects cells from CoV infection. Our study has identified the host cellular pathway hijacked for supplying CoV replication membranes and describes an autophagy-independent role for non-lipidated LC3-I.

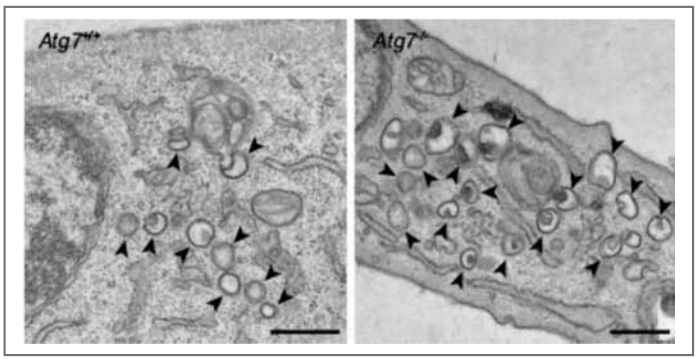


Figure 5
Coronavirus infection induces formation of ER-derived vesicles (arrowheads) both in normal and in autophagy-deficient cells. Bar, 500 nm.

Novel Approaches to Inhibit Production and Deposition of the Toxic Ab Peptide

Siro Bianchi, Carmela Galli 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 (Paganetti et al 2005).

When added extracellularly, they associate with surface exposed APP similarly interfering with Aβ production (unpublished).

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 (Figure 6). Surrounding genetically engineered C2C12 cells producing mAbs and/or antibody fragments with a synthetic permoselective membrane minimizes immunological responses by avoiding

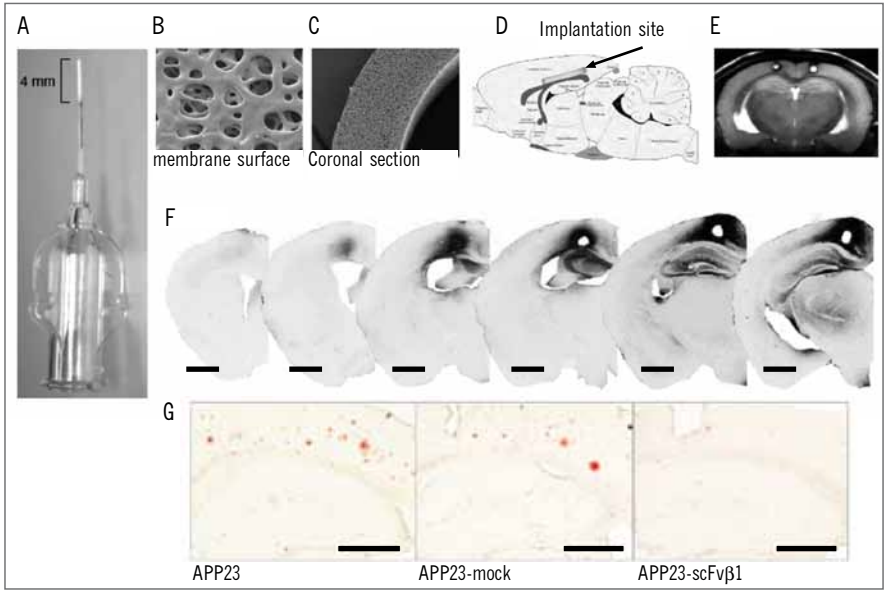


Figure 6

Polysulfone (PS) hollow fiber capsule use in mice brain implantation. (A) Macroscopical appearance of a PS hollow fiber capsule. A 4 mm PS membrane glued and connected to a stainless steel tip facilitates the loading of C2C12 cells. The plastic hub serves for introducing the Hamilton syringe containing the cells into the polymer capsule. (B) The outer surface of the PS-membrane imaged with a SEM microscope at x10,000 reveals its porosity corresponding to a cut-off of ~100 kDa. Scale bar 2 mm. (C) Coronal section of the PS-membrane at x1000 shows an even homogeneous wall structure. Scale bar 20 mm. (D) Sagittal schematic representation of the implantation site following surgery in the APP23 mice. (E) MRI coronal image showing the placement of the polymeric cell implants following bilateral implantation in the APP23 brain cortex just above the hippocampus. (F) Immunohistochemical coronal sections of APP23-scFvb1 revealing the extent of diffusion of scFvb1 around the site of implantation. Magnification 1x, scale bar 1 mm. (G) Representative congophilic stained sections of Ab plaques from brains of APP23, APP23-mock and APP23-scFvb1. Magnification 2x, scale bar 500 mm.

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

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.

Funding

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  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
  2002-2013
- *SNC of Competence in Research on Neural Plasticity and Repair*
  Project Center 2/Alzheimer's Disease
  2001-2010
- *S. Salvatore Foundation*
  Functional characterization of endoplasmic reticulum-associated protein degradation regulators implicated in tumor progression
  2005-2012
- *ONELife Advisors*
  Biogenesis of proteins involved in the Alzheimer's disease
  2010-2011
- *Association Française contre les Myopathies*
  Processing of disease-causing sarcoglycan mutanMNM2Physiopath/2010-2011

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  Uni-Zurich (CH)

Publications

- ***N-glycan structures: recognition and processing in the ER.***
  Aebi, M., R. Bernasconi, S. Clerc, and M. Molinari.
  Trends Biochem Sci. 2010; 35:74-82.
- ***ERAD substrates: which way out?***
  Hebert, D. N., R. Bernasconi, and M. Molinari.
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- ***Stringent requirement for HRD1, SEL1L, and OS-9/XTP3-B for disposal of ERAD-LS substrates.***
  Bernasconi, R., C. Galli, V. Calanca, T. Nakajima, and M. Molinari.
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- ***Coronaviruses Hijack the LC3-I-positive EDEMosomes, ER-derived vesicles exporting short-lived ERAD regulators, for replication.***
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  Cell Host Microbe. 2010; 7:500-508.
- ***Autophagy-independent LC3 function in vesicular traffic.***
  de Haan, C. A., M. Molinari, and F. Reggiori.
  Autophagy. 2010; 6:994-996.

- ***Cyclosporine a-sensitive, cyclophilin B-dependent endoplasmic reticulum-associated degradation.***
  Bernasconi, R., T. Solda, C. Galli, T. Pertel, J. Luban, and M. Molinari.
  PLoS One. 2010; 5:e13008.
- ***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.
- ***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.
- ***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.

Lectures and Seminars

- ***Basic Virology Course, Institut Pasteur***
  The folding of viral glycoproteins in the endoplasmic reticulum
  Paris (FR) / 3.9.2009
- ***Glycan Biosynthesis and Function***
  Oligosaccharide processing in protein folding and quality control
  Oulu (FI) / 17-18.9.2009
- ***CSF European Autophagy Conference 2009***
  ERAD tuning: segregation and rapid turnover of select ER-resident chaperones modulates standard ERAD and folding activities
  Ascona (CH) / 18-22.10.2009
- ***Giornate Autogestite Liceo di Lugano***
  Il ripiegamento, il controllo della qualità e la degradazione di proteine nel reticolo endoplasmatico
  Bellinzona (CH) / 9.3.2010
- ***First IRB-INGM Meeting***
  Protein folding and quality control in the mammalian endoplasmic reticulum
  Bellinzona (CH) / 18.3.2010
- ***Giornate Autogestite Liceo di Bellinzona***
  La malattia di Alzheimer
  Bellinzona (CH) / 22.3.2010
- ***Molecular Chaperones and Stress Responses***
  Substrate-specific requirements for efficient disposal from the mammalian ER
  Cold Spring Harbor (USA) / 4-8.5.2010
- ***4th Microbiology and Immunology Introductory Course***
  How Coronaviruses hijack the ERAD tuning machinery for replication
  Zurich (CH) / 7-11.7.2010
- ***25th International Carbohydrate Symposium 2010***
  Sugar processing and recognition in protein quality control
  Tokyo (JP) / 1-6.8.2010
- ***Basic Virology Course, Institut Pasteur***
  How Coronaviruses hijack the ERAD tuning machinery for replication
  Paris (FR) / 2.9.2010
- ***10th Anniversary of the Swiss Federal Office of Police***
  The Alzheimer's Disease
  Bern (CH) / 21.10.2010
- ***Meeting Beata Vergine Hospital***
  Protein Folding in the ER
  Bellinzona (CH) / 16.11.2010
- ***Institute Seminar, EPFL Lausanne***
  How Coronaviruses hijack ERAD tuning for replication
  Lausanne (CH) / 18.11.2010

Silvia Monticelli
Molecular Immunology


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Silvia Monticelli

Silvia Monticelli earned a Ph.D. in Biology at the University of Milan where she specialized in Molecular Biology. From July 2000 to January 2007 she was a post-doc in Anjana Rao's laboratory at the Center for Blood Research, Harvard Medical School in Boston (USA), and in February 2007 she joined the Institute for Research in Biomedicine in Bellinzona as Group Leader. Dr. Monticelli has published several papers covering various aspects of the molecular mechanisms underlying the response and differentiation of immune cells involved, among other processes, in allergy and asthma. Recently she focused her research efforts on the role of microRNAs, a relatively new class of regulatory molecules, in the development and function of cells of the immune system.

Research Focus

MicroRNAs (miRNAs) are a family of small non-coding RNAs that have emerged as key post-transcriptional regulators in a wide variety of organisms. Because each miRNA can regulate expression of a distinct set of genes, miRNA expression can shape the repertoire of proteins that are actually expressed during development, differentiation or disease.

The generation of the immune system from hematopoietic stem cells involves ordered events of lineage commitment, differentiation, proliferation and cell migration; within such processes, miRNAs appear ideally suited to rapidly adjust protein concentrations. Accordingly, some miRNAs are expressed in a stage-specific fashion, and miRNA control has recently emerged as a critical regulatory component in the mammalian immune system. Indeed, 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 study the role of miRNAs in the differentiation of cells of the immune system, with a special focus on T lymphocytes and mast cells. Besides being of fundamental relevance to our understanding of cell differentiation and gene regulation, elucidation of the molecular mechanisms that control mast cells and T helper cell differentiation and activation have substantial potential for clinical application in the treatment of asthma, allergy, autoimmunity, chronic inflammation and malignancies.

Team

Group Leader: Silvia Monticelli, PhD › *silvia.monticelli@irb.usi.ch*

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

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

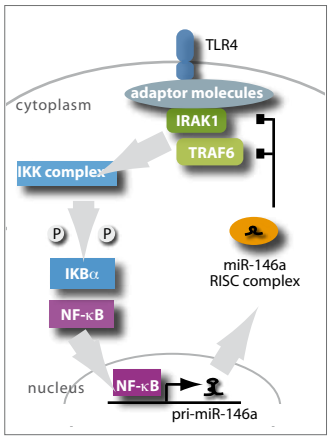
Nicole Rusca, Lorenzo Deho' and Silvia Monticelli

Mice that lack the p50 subunit of the transcription factor NF-κB 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 asked 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 p50-deficient mice were able to differentiate *in vitro* with no obvious gross phenotypic defect, including cell shape, size and granularity. BMMCs lacking p50 degranulated normally and produced cytokines similarly to wild-type cells in response to different stimuli. However, in the absence of p50, BMMCs showed increased proliferation and a strong resistance to apoptosis in response to withdrawal of essential cytokines. The combination of increased proliferation and reduced apoptosis is likely the basis for a marked increase in the number of tissue mast cells that we observed in p50-deficient animals.

MicroRNAs (miRNAs) are short RNA molecules that can potentially regulate all aspects of a cell and often depend transcriptionally on the same factors that regulate protein-coding genes. A dysregulation in miRNA expression could therefore contribute to the phenotype observed in the absence of p50 in mast cells. By further investigating the role of p50 in mast cell apoptosis, we found that BMMCs lacking p50 were severely impaired in their ability to induce expression of miR-146a, and that this miRNA plays an important role in regulating 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 p50 and miR-146a in regulating cell survival in mast cells. Moreover, miR-146a upregulation contributes by negatively regulating NF-κB activation through the down-modulation of the signalling molecules IRAK1 and TRAF6 (Figure 1).

In conclusion, mast cells lacking p50 show increased proliferation, survival and tissue accumulation, and overall normal cytokine production. While the asthma-resistant phenotype of p50-deficient animals remains primarily Th2-dependent, we identified a novel molecular network that regulates mast cell proliferation, survival and homeostasis in the tissues, which could be important in diseases related to abnormal accumulation of mast cells such as mastocytosis.



miR-146a negatively regulates signal transduction pathways leading to NF-κB activation. *Upon activation of a cell surface receptor such as TLR4, a molecular cascade including TRAF6 and IRAK1 leads to IkBα phosphorylation and degradation, followed by NF-κB activation and nuclear translocation. NF-κB activation induces transcription of many genes, including the primary transcript for miR-146a (pri-miR-146a). Once translocated to the cytoplasm and loaded onto the RISC complex, mature miR-146a contributes to attenuate receptor signalling through the down-modulation of IRAK1 and TRAF6.*

Influence of PLZF and miR-221-222 in mast cell differentiation and function

Ramon J Mayoral and Silvia Monticelli

Mast cells are cells of the innate immune system that reside in most tissues and derive from hematopoietic precursors in the bone marrow. We analyzed the expression pattern of several miRNAs in murine mast cells differentiated *in vitro*, and we identified a family of two miRNAs, miR-221 and miR-222, that were transcriptionally upregulated upon acute stimulation of differentiated mast cells, pointing towards a potential role for these miRNAs in regulating mast cell effector functions. To identify potential cis-regulatory regions that might give us insights into the mechanisms of miR-221-222 transcriptional regulation in mast cells, we analyzed the pattern of DNase I hypersensitivity (HS) in the miR-221-222 genomic locus, and we identified some conserved, mast cell-specific, accessible regions upstream of the miR-221-222 sequences. Bioinformatics analysis for putative transcription factor binding sites in these regions identified motifs that were over-represented in the DNaseI HS sites as compared to promoter sequences in the entire mouse genome, and among these we identified the transcriptional repressor PLZF. While forced expression of PLZF in hematopoietic progenitors potentiated mast cell differentiation, this effect was unrelated to miR-221-222 expression. These activation-induced miRNAs therefore regulate mast cell functions but not their differentiation. To examine the role of these miRNAs in differentiated mast cells, we developed robust lentiviral systems for miRNA overexpression and depletion. Although both miR-221 and miR-222 share the same seed sequence and potentiated mast cell adherence equivalently, miR-222-overexpressing cells showed a striking increase in mast cell migration to the peritoneal cavity and mesenteric membranes in mice, compared to cells overexpressing miR-221. In contrast, miR-221-overexpressing cells were more effective at degranulating in response to IgE-antigen complexes. Transcriptional profiling of miR-221-overexpressing cells revealed modulation of many transcripts in mast cells, including several associated with the cytoskeleton. Indeed, miR-221 and miR-222 overexpression was associated with reproducible increases in cortical actin. Our studies provide new insights into the roles of PLZF and miR-221-222 in mast cell biology, and identify novel mechanisms that may be dysregulated in pathological conditions.

» ***Mayoral R. et al.*** *J. Immunol. 2009, 182:433-445.*

» ***Mayoral R. et al.*** *Methods Mol Biol. 2010, 667:205-14.*

Funding

- *Swiss National Science Foundation*
  Role of microRNAs in the development and function of cells of the immune system
  3100A0_121991/1
  2009-2011
- *Fondazione della Banca del Ceresio*
  2008-2010

Collaborations

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  Istituto Clinico Humanitas, Rozzano, (I)

Publications

- ***Human mast cells and mastocytosis: harnessing microRNA expression as a new approach to therapy?***
  Deho, L., and S. Monticelli.
  Arch Immunol Ther Exp (Warsz). 2010; 58:279-286.
- ***Stable Overexpression of miRNAs in Bone Marrow-Derived Murine Mast Cells Using Lentiviral Expression Vectors.***
  Mayoral, R. J., and S. Monticelli.
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Book chapters

- ***'MicroRNA and the immune system: Methods and Protocols', Edited by Silvia Monticelli, Methods in Molecular Biology series, Humana Press, USA. Volume 667, Sept. 2010.***
- ***Monticelli S. MicroRNAs in hematopoietic development. In: Small, non-coding RNAs in development and cancer, Edited by Frank Slack, Imperial College Press, UK, Sept. 2010***

Lectures and Seminars

- *Helmholtz Zentrum München*
  German Research Center for Environmental Health, Institute of Molecular Immunology
  Munich (DE) / 15.07.2010
- ***'Italian Registry for Mastocytosis' Workshop***
  Hematology Clinic, University of Pavia
  Pavia (IT) / 7.05.2010
- ***'Genetic And Epigenetic Programs In Adaptive Immunity'***
  Stresa (IT) / 17.03.210
- *RNAi Europe*
  Berlin (DE) / 17-18.09.2009
- *Keystone Symposium*
  'MicroRNA and Cancer'
  Keystone (USA) / 11.06.2009

Federica Sallusto
Cellular Immunology

Federica Sallusto

Federica Sallusto received the degree of Doctor in Biology from the University of Rome and performed postdoctoral training at the italian Institute of Health in Rome (ISS) and at the Basel Institute for Immunology (BII). In two groundbreaking papers published in 1994 and 1995, she reported that monocytes are precursors of DCs and showed that DC maturation could be induced by microbial stimuli. In 1997, she became member of the BII 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 function. Since 2000, she is a group leader at the IRB. Among her recent contributions are the epigenetic control of cytokine gene expression in human T cells and the characterization of Th17 and Th22 cells. To complement her work in the field of human immunology, she started a line of research to study in vivo lymphocyte migration in the mouse system. These studies have challenged current dogma by identifying new mechanisms of lymphocyte migration in inflamed lymph nodes and in the brain. She received the Pharmacia Foundation Award in 1999, the Behring Lecture Prize in 2009, and the Award of the Foundation for Study of Neurodegenerative Diseases in 2010. Since 2010 she is member of the German Academy of Science Leopoldina.

Research Focus

Our work has been focused on the understanding of the mechanisms that control T cell priming and commitment to different fates and that regulate, in a coordinate fashion, effector functions and migratory capacities. These questions are addressed primarily in the human system, where we combine ex vivo analysis of memory T cell subsets identified by the expression of chemokine receptors with in vitro priming experiments to dissect the mechanisms of T cell polarization. This approach has recently led to the identification of chemokine receptors expressed in human Th17 and Th22 cells, the definition of their antigenic specificities and the signals required for their differentiation. In parallel, we have used the mouse system to address fundamental questions in the regulation of lymphocyte trafficking during inflammation and in autoimmunity. We also developed a method for the analysis of human naïve and memory CD4 and CD8 repertoires based on high throughput cellular screenings of expanded T cell libraries.

Team

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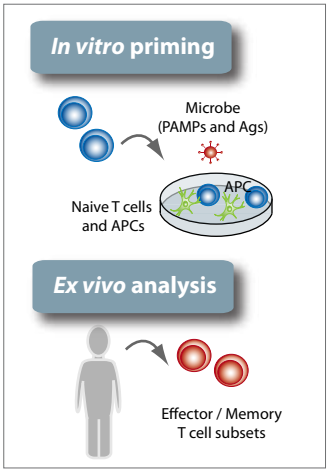
Monocytes as antigen presenting cells for the induction of T cell responses to microbes

Simone Becattini, Antonio Lanzavecchia and Federica Sallusto



Monocytes represent about 10% of blood leukocytes in humans and have a fundamental role as precursors of cells involved in tissue homeostasis, inflammation, antigen presentation and pathogen clearance. Monocytes were shown to give rise to dendritic cells, the professional antigen presenting cells, both *in vitro* and *in vivo*. Yet it is currently not clear what is the relative contribution of monocytes in the induction of adaptive immune responses as compared to dendritic cells that are found in blood, peripheral tissues or secondary lymphoid organs. We are investigating the role and function of human monocytes in priming human T cell responses to microbes *in vitro*. First, we are studying the response of monocytes to different microbes in terms of modulation of surface marker expression, cytokine production, and regulation of gene transcription. Second, we are using an *in vitro* T cell priming system wherein CFSE-labelled naive $CD4^+$ T cells are cultured with monocytes and whole microbes, in the absence of exogenous cytokines. The latter approach would take advantage of the complexity of the microbe, which provides, on the one hand, large numbers of T cell epitopes for stimulation of antigen-specific naïve T cells and, on the other hand, a variety of stimuli that trigger innate receptors on antigen presenting cells, leading to the production of cytokines and other T cell differentiation cues (Figure 1).

← *Figure 1: A combined approach to study T cell responses in humans.*
In in vitro priming, human naïve T cells are isolated and challenged in cell culture with various microbial stimuli and antigen presenting cells (APCs) and T cell polarization is analysed after activation. Using ex vivo analyses, effector/memory T cells, which have been generated through natural infection in the human host, are isolated and subsets are characterized.

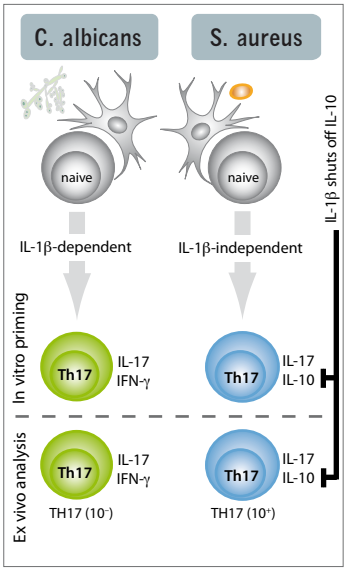
Reciprocal regulation of IFN-γ and IL-10 in human microbe-specific Th17 cells

Christina E. Zielinski, David Jarrossay, Francesca Ronchi, Antonio Lanzavecchia and Federica Sallusto

Th17 cells represent a distinct lineage of $CD4^+$ T helper cells characterized by the production of IL-17A, IL-17F and IL-22 that are involved in autoimmunity as well as in host defence. The differentiation requirements and properties of Th17 cells have been extensively investigated in the context of autoimmunity. Initial studies showed that IL-6 and TGF-β induce early Th17 differentiation, which is reinforced by IL-23 leading to acquisition of pathogenic activity. Subsequent studies revealed that, in the absence of TGF-β signalling, IL-6, IL-23 and IL-1β can directly drive differentiation of pathogenic autoreactive Th17 cells. The requirements for differentiation and the properties of pathogen-induced Th17 cells remain, however, poorly defined. Using a microbe-driven T cell priming assay, we found that C. albicans (C.a.) primes human IFN-γ-producing Th17 cells in an IL-1β-dependent fashion,

while S. aureus (S.a.) primes IL-10-producing Th17 cells in an IL-1β-independent fashion. IL-10 production was observed in recently activated Th17 cells, concomitantly with down-regulation of RORγt expression and IL-17 production. This transient anti-inflammatory switch was a characteristic of both *in vitro* primed and ex vivo isolated memory Th17 cells. The activation-dependent production of IL-10 was enhanced by IL-12, IL-23, and IL-27, and dominantly suppressed by IL-1β. In patients with overproduction of IL-1β, treatment with an IL-1RI antagonist led to increased IL-10 expression by Th17 cells, supporting the notion that IL-1β plays a critical role in regulating IL-10 production by Th17 cells. These findings demonstrate that C.a. and S.a. induce distinct types of Th17 cells that differ in priming requirements and effector function (Figure 2) and shed light on immune mechanisms of adaptive immunity that may help the development of vaccine strategies against these pathogens.

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



➜ ***Figure 2: Two types of human Th17 cells and the role of IL-1b.***
Results of in vitro human T cell priming assays using C. albicans and S. aureus and ex vivo analysis with human memory Th17 cells. IL-1b regulates priming of microbe-specific Th17 cells and IL-10 production.

Human memory T cell responses to microbes: specificity and function

Federico Mele and Federica Sallusto

Memory T and B cells represent a repository of the antigenic experience of the individual. Heterogeneity is a hallmark of memory T cells (Figure 3). Defining the distribution of antigen-specific memory cells in different memory compartments can provide useful information on the *in vivo* response following infection or vaccination. We are performing a systematic analysis of the frequency, distribution, and fine specificity of T cells specific for different microbes in naïve, effector and memory T cell subsets. To study the antigenic repertoire of memory T cell subsets, Th1, Th2, Th17, Th22 and other memory T cell subsets are isolated from peripheral blood according to expression of CD45RO and chemokine receptors. T cell libraries from these subsets are generated and interrogated simultaneously for reactivity against a panel of antigens from viruses (e.g. CMV, HBV, Influenza virus), bacteria (e.g. *Mycobacterium tuberculosis*, *Staphylococcus aureus*) and fungi (e.g. *Candida albicans*). Since different classes of molecules are involved in the presentation of microbial antigens to $CD4^+$ T cells, namely MHC-II (for peptide antigens) and CD1-family (for lipid antigens), we will perform the screening using either monocytes (MHC class $II^+CD1a^-CD1b^-CD1c^-$) or monocyte-derived DCs (MHC class $II^+CD1a^+CD1b^+CD1c^+$, Sallusto, 1994) in order to study the distribution of T cells specific for protein or lipid antigens (Barral and Brenner, 2007). 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 cytokines, in particular IFN-γ, IL-4, IL-5, IL-13, IL-17, IL-22, and IL-10. We expect to gain important information on the distribution, frequency and class of the human T cell response to different pathogens and in different stimulatory conditions (natural infection or vaccination).

T CELL	DIFFERENTIATION SIGNAL	TX FACTOR	EFFECTOR MOLECULE(S)	HOMING RECEPTORS	TARGET CELLS	FUNCTION
Th1	IL-12,IFN	T-bet	IFN-γ	CXCR3	Macrophages	Bacteria
Th2	IL-4	GATA-3	IL-4,-5,-13	CCR4/CRTh2	Eosinophils	Parasites
Th17	IL-6,-1β,TGF-β	ROR-γt	IL-17,-22	CCR6+CCR4	Neutrophils	Fungi
Th9	IL-4,TGF-β	?	IL-9	?	Mast cells	Helminths
Th22	IL-6,TNF	AHR?	IL-22	CCR10+CCR6	Epithelia	Skin protect?
TFH	(IL-21)	Bcl-6	IL-21	CXCR5	B cells	Antibodies
CTL	IL-12,CD4	Eomes	Perforin	CXCR3/CCR5	Tissue cells	Viruses
Treg	(TGF-β)	FOXP3	TGF-β	CCR7/CCR6	DC/T cells	Regulation
Tr1	IL-10	?	IL-10	CX3CR1	T cells	Regulation

Figure 3: T cell-mediated immune responses: heterogeneity and specialization.
Differentiation signal(s), transcription (TX) factor usage, effector molecule(s), homing receptors, target cells and function of each T cell subset.

Differential expression of chemokine receptors among human regulatory T cells

Thomas Duhen, Rebekka Geiger and Federica Sallusto

Human memory $CD4^+$ T cell subsets have been defined by their chemokine receptor expression profile enabling them to migrate to specific tissues. We hypothesized that regulatory T (Treg) cells may also display different patterns of chemokine receptors. $CD4^+CD25^{hi}CD45RA^-$ Treg cells from peripheral blood of healthy donors were analyzed for their expression of CCR6, CCR4 and CCR10. As previously described, a vast majority of Treg cells express CCR6. Among the $CCR6^+$ cells, Treg cells can be separated into two main subsets on the basis of CCR4 and CCR10 expression. $CCR4^+CCR10^+$ were present in high proportions, while a minor subset comprised $CCR4^+CCR10^-$ cells. Treg subsets were analyzed by intracellular staining for Foxp3 expression and cytokine production upon stimulation with PMA + ionomycin. $Foxp3^+IL\text{-}10^+$ Treg cells were detected in the $CCR6^-$ and $CCR6^+CCR4^+CCR10^-$ subsets, although the proportion of $IL\text{-}10^+$ cells was significantly higher in the latter. $Foxp3^+IL\text{-}17^+$ cells were present only in the $CCR6^+CCR4^+CCR10^-$ Treg

subset. In contrast, the CCR10$^+$ Treg subset did not produce any of the tested cytokines. We next examined the capacity of the Treg subsets to suppress proliferation of CFSE-labelled autologous CD4$^+$CD25– T cells stimulated by monocytes and anti-CD3. All subsets were equally potent in suppressing the proliferation of CD4$^+$CD25– T cells in a dose dependent manner. These results reveal that subsets of conventional memory T cells that differ in chemokine receptor expression and cytokine production have a counterpart in Treg subsets. Interestingly, the proportion of Treg cells with skin-homing properties is larger than the corresponding population of memory T cells. Hence, a vast majority of Treg cells in human peripheral blood appears to be specialized in the regulation of local immune responses in the skin.

This work in now being completed in collaboration with Daniel J. Campbell, Benaroya Institute, Seattle, USA.

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. The most recent studies demonstrate that CCR6 is expressed on the newly described T helper 17 (Th17) cell subset, and that 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 cross epithelial cells of the choroid plexus, thus entering the CNS and initiating brain inflammation that leads to 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 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 a library of at least 2.5 x 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 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 novel therapeutic approaches in MS based on the blockade of CCR6. Currently these studies are extended to inflammatory skin diseases by analysing the distribution of autoreactive T cells in pemphigus.

This project is done in collaborations with Antonio Uccelli (Neuroimmunology Unit, University of Genoa), Giovanna Zambruno (Dermatology Institute IDI, Rome), Michael Hertl (Department of Dermatology, University of Marburg), and Bruno Kiewski (University of Heidelberg).

Longitudinal analysis of the immune response of human CD4+ T cells to an influenza vaccine

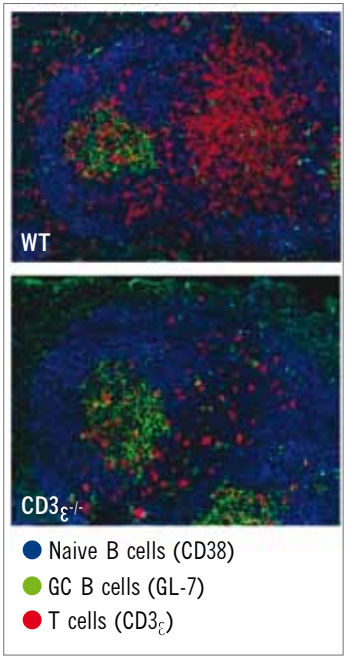
Tess Brodie, Claudia Ferlito and Federica Sallusto

Upon infection or vaccination, dendritic cells present antigen and stimulate rare antigen-specific $CD4^+$ T cells. Some primed T cells migrate to secondary lymphoid follicles to become T follicular helper cells (T_{FH}), which specialize in aiding B cells to produce antibodies and to become high affinity plasma cells, while others migrate to peripheral sites of antigen exposure to fight pathogens as the appropriate effector T cells. After antigen clearance two major types of memory T cells remain in the blood: central memory cells (TCM) that patrol lymphoid organs, and effector memory cells (T_{EM}) that patrol peripheral tissues. A subset of memory T_{FH} cells can also be found in peripheral blood (defined here cT_{FH}) but their relationship to T_{FH} in lymphoid follicles remains to be determined. Our aim is to study influenza-responding cells in diverse human T cell subsets over a vaccine time course and to gain insights in the nature of cT_{FH} cells. Using the $CD4^+$ T cell library method developed in the laboratory, we are comparing, in different donors, the repertoire of naïve (T_N), cT_{FH}, T_{CM}, and T_{EM} cells before and seven and 15 days after an immune challenge with Influvac, a seasonal influenza vaccine. From the same donors we are generating HA-specific EBV immortalized B cells, thus providing an unlimited source of APCs. Pre-vaccination, cT_{FH}, TCM, and T_{EM} subsets had a similar frequency of antigen responding cells, but seven days after vaccination the number of antigen-specific cells in cT_{FH} and T_{EM} subsets increased, while the TCM frequency remained the same. Fifteen days post-vaccination there was a dramatic increase in the number of TCM responding cells, while cT_{FH} and T_{EM} decreased moderately. Beside changes in frequency of specific T cells, we found an evolution in the avidity of T cells after vaccination. Similar to mouse T_{FH} cells in B cell follicles, cT_{FH} cells had a trend of higher antigen avidity at day 7 and a different repertoire compared to the other subsets. Our future direction consists of further characterizing the cT_{FH} subset and comparing the cT_{FH} response to the T_{FH} response in terms of Bcl-6, ICOS and PD-1 expression. Taken together these preliminary results support the notion that cT_{FH} cells are distinct from TCM and T_{EM} T cell subsets, and have characteristics similar to T_{FH} in lymphoid tissues. While it is too early to establish a connection between cT_{FH} cells and T_{FH} cells, this data gives us impetus to continue researching in this direction. As a final endpoint, we hope to determine if the quantity and characteristics of antigen-specific cT_{FH} cells can be used as an immune correlate, or as a predictor of antibody responses.

Dysregulated T follicular helper cells cause hypergammaglobulinemia and autoimmunity after immunization in T cell lymphopenic environments

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

Cognate interactions between T helper cells and B cells are required for the production of high-affinity antibodies and for the generation of memory B cells and long-lived plasma cells. Here, we studied T-dependent antibody responses under T cell lymphopenic conditions and found that after immunization, adoptively transferred antigen-specific $CD4^+$ T cells preferentially differentiated into T follicular helper (T_{FH}) cells (Figure 4). Although initiation of germinal center (GC) reaction and differentiation of B cells into antibody-secreting short-lived plasma blasts were normal, T_{FH} cells became dysfunctional and failed to provide specific B cell help to OVA-specific B cells during late GC events, resulting in impaired affinity-maturation and lack of long-lived plasma cells, and failed to induce recall responses. Instead, hypergammaglobulinemia, hyper-IgE, and increased production of autoantibodies were observed in the recipients. These results suggest a previously unappreciated role for antigen-specific T cells in mediating polyclonal bystander activation while at the same time compromising antigen-specific immune responses, ultimately resulting in overt autoimmunity. We are currently addressing the role of cytotoxic T cells and regulatory T cells in the observed phenomena. We are also investigating the B cell behaviour in mice adoptively transferred with OT-II T cells and HEL-specific B cells (SWHEL) following immunization with a recombinant protein in which HEL is fused in frame with the OVA323-339 peptide.



↗ ***Figure 4: T cells and germinal centres are detected in immunized OT-II/CD3e$^{-/-}$ mice.***
Wild-type (WT) and CD3e$^{-/-}$ mice were adoptively transferred with OVA-specific OT-II cells and immunized with OVA/alum intraperitoneally. Germinal centre (GC) formation was analysed in the spleen on day +10. Naïve B cells were stained with antibodies against CD38 (blue), GC B cells with GL-7 (green) and T cells with CD3e (red). Magnification, 150x.

Kinetics of T lymphocytes priming with dendritic cells in vivo

Margot Cucchetti and Federica Sallusto

The inflammatory environment of secondary lymphoid organs differ during the course of an infection or vaccination, suggesting that these variables may affect the fate of the primed T lymphocytes. We investigated the fate of TCR transgenic $CD4^+$ T cells when activated at high (i.e. in the early phases of an immune response) or low (at later time points of an immune response) stimulatory conditions. We found that at high stimulatory conditions naive $CD4^+$ T cells are efficiently activated and proliferate vigorously, both *in vitro* and *in vivo*. At low stimulatory conditions however, both $CD4^+$ and $CD8^+$ T cells proliferate less and divide slower compared to $CD4^+$ and $CD8^+$ T cells primed in the early phases of the immunization. Notably, late primed $CD4^+$ T cells partially undergo apoptosis in the lymph nodes, while a

few migrate to the bone marrow. Instead, $CD4^+$ and $CD8^+$ T cells primed in the early phases of the immunization can be found in spleen and bone marrow. These studies may provide new information on T cell fate determination and T cell memory generation.
Margot Cucchetti received the Master at the Ecole Supérieure de Biotechnologie de Strasbourg (ESBS), Illkirch (FR). Thesis: "Kinetics of T lymphocytes priming with dendritic cells *in vivo*".

On the role of IL-1β and CNS myeloid cells in EAE pathogenesis

Francesca Ronchi, Camilla Basso, Andrea Reboldi and Federica Sallusto

IL-1β is a pleiotropic cytokine produced following cleavage of pro-IL-1β by interleukin-1 converting enzyme (caspase-1), which in turn is activated by the inflammasome. This cytokine has been shown to play a role in several inflammatory disorders in humans and in experimental animal models, including mouse experimental autoimmune encephalomyelitis (EAE). IL-1β has also 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 IFN-γ. We found that mice deficient 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 MOG in CFA and pertussis toxin (PT). Autoreactive T cells were primed in both wild-type and $ASC^{-/-}$ mice. However, these cells expanded poorly in $ASC^{-/-}$ mice and showed reduced capacity to produce simultaneously several inflammatory cytokines, such as IL-17, IL-22, IFN-γ, and GM-CSF. The induction of multifunctional (IL-17+ IL-22+ IFNγ+ GM-CSF+) T cells in wild-type mice, but not $ASC^{-/-}$ mice, was dependent on the presence of PT at the time of immunization and was observed using different adjuvants. PT was found to induce IL-1β secretion from antigen-presenting cells, such as DCs and microglia. These data suggest that the disease-inducing effect of PT may be due to its ability to induce production of IL-1β, which in turn triggers differentiation of pathogenic multifunctional T cells. In addition to its effect in the induction phase, we are testing whether IL-1β, or other inflammasome-generated cytokines, may be required in the effector phases of EAE. We found that $ASC^{-/-}$ mice are resistant to passive EAE induction. In addition, bone marrow chimera experiments of mice transplanted with bone marrow cells from wild-type mice or $ASC^{-/-}$ mice showed that IL-1β production by microglial cells, CNS-resident myeloid cells and CNS-migrating inflammatory monocytes is important for EAE pathogenesis. These results are consistent with the notion that microglial cells express proteins of the inflammasome complex and with our finding that these cells release active IL-1β upon stimulation with inflammatory cytokines or TLR agonists *in vitro*.

Funding

- *Swiss National Science Foundation*
  Memory T cell subsets in man and mouse: identification, characterization and function
  3100A0-116440 / 2007-2010
- *European Union*
  INTEGRAM: International graduate programme in molecular medicine
  Project no. 20386 / 2006-2010
- *European Union*
  DC-Thera: Dendritic Cells for Novel Immunotherapies
  Project no. 512074 / 2005-2010
- *European Union*
  Sens-it-iv: Novel Testing Strategies for *In Vitro* Assessment of Allergens
  Project no. 018681 / 2005-2010
- *European Union*
  Innochem: Innovative Chemokine-based Therapeutic Strategies for Autoimmunity
  Project no. 518167 / 2005 – 31.10.2010
- *Bill and Melinda Gates Foundation*
  PTVDC: Pox T Cell Vaccine Discovery Consortium / 2006-2011
- *European Union*
  Pemphigus: From autoimmunity to disease
  Project no. 200515 / 2008-2011
- *Gottfried and Julia Bangerter Rhyner Foundation*
  Isolation of human monoclonal antibodies to understand Dengue virus neutralization and enhancement / 2008-2010
- *Karitative Stiftung Dr. Gerber-ten Bosch*
  2008-2009
- *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 / 2010-2013
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  Effector and memory T cell subsets in man and mouse
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Collaborations

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- *Antonio Uccelli*
  University of Genova, Genova (IT)

Publications

- ***Heterogeneity of CD4⁺ memory T cells: functional modules for tailored immunity.***
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- ***Isolation of human monoclonal antibodies that potently neutralize human cytomegalovirus infection by targeting different epitopes on the gH/gL/UL128-131A complex.***
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- ***From vaccines to memory and back.***
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Lectures and Seminars

- *Keystone Symposium*
  "Mobilizing Cellular Immunity for Cancer Therapy"
  Snowbird, UT (USA) / 11-16.01.2009
- *Harvard Medical School*
  Seminar "Lymphocyte trafficking in immunity and autoimmunity"
  Boston, MA (USA) / 04.02.2009
- *Massachusetts General Hospital*
  Seminar "Human memory T cells: heterogeneity, stability and flexibility"
  Boston, MA (USA) / 05.02.2009
- *Keystone Symposium*
  "TH17 Cells in Health and Disease"
  Vancouver (CA) / 5-9.02.2009
- *Keystone Symposium*
  "Immunologic Memory and Host Defense"
  Keystone, CO (USA) / 08-13.02.2009.
- *Accademia Medica di Roma*
  Seminar "Migrazione e funzione effettrice dei linfociti T nelle patologie autoimmuni"
  Roma (IT) / 05.03.2009
- *World Immune Regulation Meeting-III*
  Davos (CH) / 21-24.03.2009
- *University of Genoa*
  Seminar "Lymphocyte trafficking in immunity and autoimmunity"
  Genoa (IT) / 22-05-2009
- *8th Elsinore Meeting on Infection Immunity*
  "Prophylactic and therapeutic intervention in host-pathogen interaction"
  Helsingoer (DK) / 05-08.06.2009
- *Tri-Society Annual Conference*
  "Cellular and Cytokine Interactions in Health and Disease"
  Lisbon (PT) / 18-21.10.2009
- *University of Marburg*
  Behring Lecture 2009
  Marburg (DE) / 25.11.2009
- *New York University School of Medicine*
  Immunology Club seminar series
  Seminar "T lymphocyte differentiation, migration and immune regulation"
  New York, NY (USA) / 10.12.2009
- *38th Annual meeting, Israeli Immunology Society*
  Haifa (IL) / 04.02.2010
- *Lecture Course on "Genetic and epigenetic programs in adaptive immunity"*
  Stresa (IT) / 15-18.03.2010
- *World Immune Regulation Meeting-IV*
  Davos (CH) / 28.03-01.04.2010
- *Cold Spring Harbor Laboratory Conference*
  "Gene Expression & Signaling in the Immune System"
  Cold Spring Harbor, NY (USA) / 21-25.04.2010
- *20th Nikolas Symposium*
  Loutraki (GR) / 30.04-03.05.2010
- *Gordon Research Conference*
  "Immunochemistry and Immunobiology"
  Les Diablerets (CH) / 16-21.05.2010
- *Novo Nordisk A/S*
  Seminar "Dissecting the heterogeneity of human memory T cells"
  Maaloev (DK) / 25.05.2010
- *University of Zurich*
  Fourth Microbiology and Immunology Introductory Course
  Microbiology and Immunology Graduate School
  Zurich (CH) / 08-12.06.2010
- *4th MASIR Conference*
  Mikonos (GR) / 09-12.06.2010
- *FASEB Summer Research Conference*
  "Biology of the immune system"
  Carefree, AZ (USA) / 20-25.06.2010

- *Gordon Research Conference*
  "Barriers in the CNS"
  New London, NH (USA) / 20-25.06.2010
- *14th International Congress of Immunology*
  Kobe (JP) / 22-27.08.2010
- *National Institutes of Health (USA)*
  National Institute of Allergy and Infectious Diseases
  Workshop: "*In Vitro*" systems to characterize the immune responses to HIV-1
  Bethesda, MD (USA) / 4.08.2010
- *University of Amsterdam*
  Academic Medical Center, Ruysch Lecture
  Amsterdam (NL) / 21.09.2010.
- *Free University Medical Center*
  Advanced Immunology Course "Advances in biology of APCs and T cells"
  Amsterdam (NL) / 22.09.2010
- *University of Cardiff*
  School of Medicine, The Jonathan Boulter Memorial Lecture 2010
- *"Towards better understanding of T cell immunity"*
  Cardiff (UK) / 21.10.2010
- *University of Cardiff*
  School of Medicine
  Seminar "Human Th17 cells"
  Cardiff (UK) / 21.10.2010
- *MACS Symposium "The T cell adaptive immune response"*
  Florence (IT) / 29.10.2010
- *University Hospital Hamburg-Eppendorf*
  Symposium: Mechanisms of autoimmune diseases
  Hamburg (DE) / 12-13.11.2010
- *Yale University School of Medicine*
  Human and Translational Immunology seminar series
  Seminar: "T lymphocyte differentiation, migration and immune regulation"
  New Haven (USA) / 07.12.2010
- *U.T. MD Anderson Cancer Center*
  Department of Immunology
  Seminar: "T lymphocytes in immunity and autoimmunity"
  Houston, TX (USA) / 09.12.2010
- *IOSI Conferenze Formative*
  Seminar "I linfociti T nelle patologie autoimmuni: dai modelli animali all'uomo"
  Bellinzona (CH) / 15.12.2010
- *Keystone Symposium*
  "Tuberculosis: immunology, cell biology and novel vaccination strategies"
  Vancouver (CA) / 15-20.01.2011
- *The University of Birmingham*
  Alumni Lecture in Immunology
  "T cell differentiation, migration, and immune regulation"
  Birmingham (UK) / 27.01.2011
- *Keystone Symposium*
  "Immunologic memory, persisting microbes and chronic disease"
  Banff (CA) / 06-11.02.2011
- *Keystone Symposium*
  "Dendritic cells and the initiation of adaptive immunity"
  Santa Fe, NM (USA) / 12-17.02.2011
- *Imperial College London*
  Seminar: "Dissecting the human T cell response to microbes"
  London (UK) / 08.03.2011

Marcus Thelen
Signal Transduction


DELL

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

Chemotactic cytokines and their cognate receptors are the most important regulators for cell migration in vertebrates. The chemokine system not only controls leukocyte trafficking during immune homeostasis, surveillance and responses, but also the positioning of somatic cells, e.g. during development, and in pathological situations such as tumor growth and spreading. By and large, cells migrate along guidance cues formed by gradients of appropriate chemokines. The latter 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.

Team

Group Leader: Marcus Thelen, PhD > *marcus.thelen@irb.usi.ch*
Members: Tiziana Apuzzo, *PhD student* – Luise Humpert, *PhD student* – Ulrike Naumann, *PhD student* – Sylvia Thelen, *PhD* – Silvia Volpe, *PhD student*

Novel CXCR4-activated signal transduction pathways

Tiziana Apuzzo and Marcus Thelen

Chemokine receptors belong to the rhodopsin-like subfamily of heptahelical G-protein coupled receptors and share the ability to mediate leukocyte migration. The ubiquitously expressed chemokine receptor CXCR4 was shown to have, in addition to its function on leukocytes, a critical role during embryogenesis and 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. Mesenchymal migration is characterized by a much slower movement than observed with leukocytes and depends on stronger adhesion sites. CXCR4 further distinguishes from other chemokine receptors through its signaling properties and functional responsiveness. Like other chemokine receptors, CXCR4 is coupled to pertussis toxin-sensitive heterotrimeric Gi-proteins, but has the ability to trigger the prolonged activation of intracellular signal transduction. Nevertheless, the presence of the receptor on a cell surface does not correlate with its activity profile. Thus, it is conceivable that CXCR4, in addition to Gi-coupling, interacts with additional proteins, which regulate the characteristic responses of the receptor. The aim of the project is to identify novel interaction partners of CXCR4 that mediate the typical receptor responses. Several laboratories used the C-terminus of a GPCR as bait in GST-pull down or yeast two hybrid experiments to identify associated proteins. However, the cytoplasmic surface of a chemokine receptor consists not only of the C-terminus, but includes three intracellular loops, which connect the transmembrane helices. These loops, together with the C-terminus, form a large structured surface to which proteins can bind. We developed a protocol which allows the immunoprecipitation of CXCR4 under mild detergent conditions which preserves the native conformation of the receptors and the association with downstream effectors. We have used mass-spectrometry to unveil several, unreported interactions of CXCR4 with cytosolic proteins. Among the proteins we identified are potential regulators of protein synthesis, which could represent a direct link between CXCR4 and *de novo* protein synthesis. The finding is in agreement with the observation that migration of tumor cells requires active protein synthesis.

G-protein coupled receptor activity in migrating cells

Silvia Volpe and Marcus 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

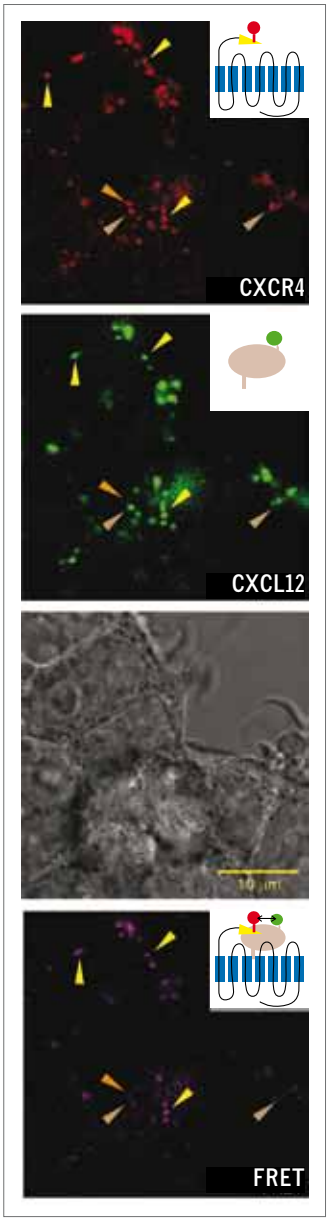
forming a characteristic leading edge and a trailing uropod. Intracellular polarization is realized by locally activated enzymes, e.g. PI 3-kinase activity is stimulated in the front whereas pathways dependent on the small GTPase Rho are activated at the rear. Leukocytes rapidly assume the typical morphology 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). Recently, receptors tagged with fluorescent probes were employed to observe the activation of GPCRs by fluorescence energy transfer (FRET). We used alpha adrenergic receptors tagged with CFP and YFP to measure their spatio-temporal activation in a chemotactic gradient by time-lapse video microscopy. Confocal section analyses reveal an equal distribution of chemoattractant receptors over the entire plasma membrane in cells moving along a chemotactic gradient. We can time resolve the activation of selective intracellular pathways at the leading edge, suggesting non-uniform signaling downstream of chemoattractant receptors. Reversion of the chemotactic gradient appears not to affect the overall receptor activity at the plasma membrane, but relocalizes downstream signaling cascades. The observations are consistent with a rapid signaling of the receptors, leading to the suppression of the activation of downstream signal transduction pathways.

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.

Molecular mechanisms of CXCR7 sorting and potential signaling properties.

Marie-Luise Humpert and Marcus Thelen

Decoy receptors for chemokines 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. Recently it



was shown that CXCR7 also controls the availability of CXCL12 during development of the central nervous system.

In general chemokine receptors were shown to interact with pertussis toxin sensitive Gi proteins. Among the various constituents of the chemokine system CXCL12 and its 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 defective 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 to the marked role in embryogenesis and regulation of hematopoiesis, the 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.

Our laboratory recently described CXCR7 as novel receptor for the chemokine CXCL12. Previous phylogenetic analyses placed the receptor in direct vicinity to chemokine receptors within the rhodopsin family of GPCRs. Nevertheless, because coupling to G-proteins and typical chemokine receptor-mediated signal transduction upon ligand binding could not be demonstrated for CXCR7, the receptor should be classified as 7TMD-receptor and not as a GPCR. Despite the lack of signaling, CXCR7 appears to play a critical role in development, because targeted deletion in mice is lethal. The failure of coupling to G-proteins led to the hypothesis that the receptor may act primarily as a scavenger. Indeed, we recently provided 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:

- *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 and fluorescent ligands, the initial steps of cargo sorting will be analyzed.

- *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.

Live HEK293 cells expressing CXCR4 labeled at the N-terminus with Atto-647N (red fluorescence) were incubated with CXCL12 biotinylated at the C-terminus conjugates with Quantum dots (QD 625) (green fluorescence) and analyzed by confocal microscopy. Fluorescence resonance energy transfer (FRET) (magenta color) occurs when CXCL12 is bound to CXCR4 (yellow arrows) revealing internalized receptor ligand complexes. Brown arrows indicate endosomes where receptor and ligand colocalize, but are not bound to each other. The orange arrow indicates internalized receptor without ligand.

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. The project aims for the molecular characterization of different P-Rex1 domains and their role in chemokine receptor-mediated signal transduction. To this end, various P-Rex1 mutants will be transduced into hematopoietic precursor cells, which are derived from animals with a genetic deletion of P-Rex1. The cells can be differentiated later into neutrophils and monocytes and tested for the activity of the respective P-Rex1 mutants. To further investigate the expression of P-Rex1 in different tissues and to better reveal its subcellular localization, we generated monoclonal antibodies suitable for immunofluorescence analysis and immunohistochemistry. Staining of normal epithelium and invasive cancer from human esophagus reveals a marked upregulated expression of P-Rex1 in the tumor. The data suggest a potential role of P-Rex1 for tumor cell migration and invasion.

Funding

- *San Salvatore Foundation*
  Analysis of chemokine receptor CXCR4 interacting proteins in different tissues
  2006-2009
- *European Union*
  INNOCHEM: Innovative chemokine-based therapeutic strategies for autoimmunity and chronic inflammation FP6 – LSHP-CT-2005-518167 / 2005-2010
- *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

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Publications

- ***Chemokine receptor oligomerization: functional considerations.***
  Thelen, M., L. M. Munoz, J. M. Rodriguez-Frade, and M. Mellado.
  Curr Opin Pharmacol. 2010; 10:38-43.
- ***CXCR7 functions as a scavenger for CXCL12 and CXCL11.***
  Naumann, U., E. Cameroni, M. Pruenster, H. Mahabaleshwar, E. Raz, H. G. Zerwes, A. Rot, and M. Thelen.
  PLoS One. 2010; 5:e9175.
- ***Polarization of migrating monocytic cells is independent of PI 3-kinase activity.***
  Volpe, S., S. Thelen, T. Pertel, M. J. Lohse, and M. Thelen.
  PLoS One. 2010; 5:e10159.

Lectures and Seminars

- *University of Lausanne, CHUV*
  Seminar"Functional chemokine receptor responses"
  Lausanne (CH) / 18. 03. 2011
- *Cytokines and Chemokines, post-transcriptional regulation*
  CXCR7 functions as scavenger for CXCL12
  Saint-Sorlin (FR) / 23-24.03.2010
- *Forefronts Symposium International Nephrology Society (INS)*
  Function of chemokine decoy receptors in tuning the immune response
  Sylt (DE) / 06-09.05.2010
- *Gordon Research Conference on Chemotactic Cytokines*
  The Yin Yang of CXCR7 functions
  Il Ciocco (IT) / 30.06-03.07.2010
- *University of Bern*
  Institute of Pharmacology
  Seminar"The Yin Yang of CXCR7 functions"
  Bern (CH) / 07.07.2010
- *Novartis Pharma*
  The Yin Yang of CXCR7 functions
  Basel (CH) / 08.07.2010

- *University of Geneva*
  Centre médical universitaire (CMU)
  Seminar "The Yin Yang of CXCR7 functions"
  Geneva (CH) / 21.07.2010
- *University of Münster, ZMBE*
  Seminar "Chemokine receptor functions"
  Münster (DE) / 08.09.2010

Mariagrazia Uguccioni
Chemokines in Immunity

Mariagrazia Uguccioni

Mariagrazia Uguccioni received a degree in Medicine from the University of Bologna (IT) where she specialized in Haematology in 1994. From 1993 to 2000 she was a member of the Theodor Kocher Institute, University of Bern (CH), and since 2000 she is group leader at the IRB. She is adjunct professor of Immunology at the School of Rheumatology, University of Bologna (IT) since 2000, and 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 autoimmune 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 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.

Team

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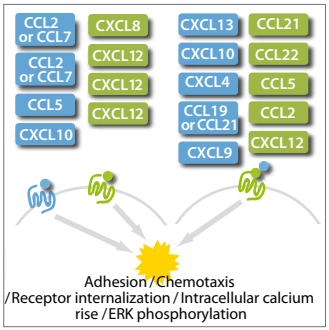
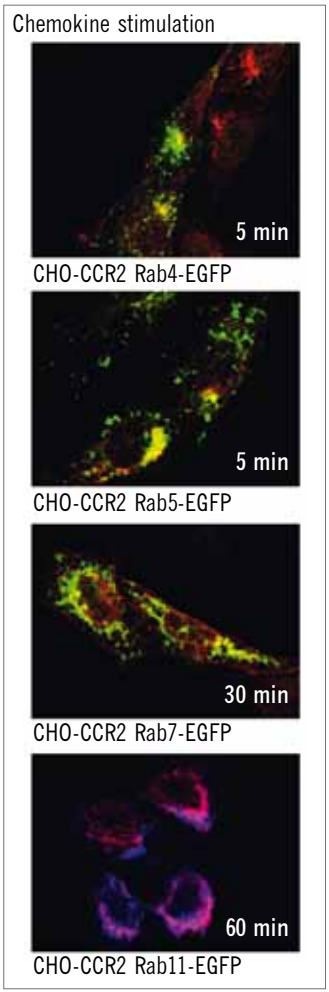
Members: Maria Gabriela Danelon, *Technician* – Daniel Venetz, *PhD student* – Milena Schiraldi, *PhD student* – Luisa Stefano, *PhD* – Denise Bottinelli, *PhD student* – Valentina Cecchinato, *PhD*

Chemokines: Structure/Function Studies

Milena Schiraldi, Luisa Stefano, 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).

We are currently dissecting the endocytic pathway involved in the trafficking of G-protein coupled receptors upon triggering with the selective agonist and in the presence of synergy-inducing chemokines by taking advantage of cells doubly transfected with a chemokine receptor and different Ras-like small GTPases (Rab GTPases) (Figure 2). These studies will shed light on the possible destinies of chemokine receptors triggered by the chemokine heterocomplexes, which could account for the diverse functional responses we have observed *in vitro*.

Not only chemokines can modulate the activity of chemokine agonists, but also molecules released during inflammation, such as HMGB1, can form complexes with chemokines and modulate their activity on the selective receptors. The molecular basis of synergism remains, at present, not fully understood and the analysis of this phenomenon is part of our ongoing research.

The chemokine CXCL12 plays an essential and unique role in homeostatic regulation of leukocyte trafficking and tissue regeneration. The chromatin protein HMGB1 is released by dying and distressed cells, and acts as a Damage Associated Molecular Pattern (DAMP) or alarmin, promoting cell migration towards the site of tissue damage. It is currently not known whether all receptors and all signalling pathways are required for the different responses to HMGB1. Recent evidence suggests that TLR4, but not RAGE, is required for cytokine release, and that RAGE is involved in cell migration. Surprisingly, HMGB1-induced cell migration requires activation of both the canonical and non-canonical NF-κB pathways, which lead to the transcription of the *Cxcl12* gene. Conversely, CXCL12 induces HMGB1 release by dendritic cells and macrophages. Finally, HMGB1 protects CXCL12 from degradation, suggesting both a functional and physical interaction between the molecules. Functional as well as structural studies are being performed to study how HMGB1 and CXCL12 cooperate in promoting cell migration.

↖ ***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.*

← ***Figure 2:*** *Agonist-stimulated co-localization of CCR2 with Rab proteins in CCR2⁺Rab⁺ CHO cells.*

Inflammation and tumour

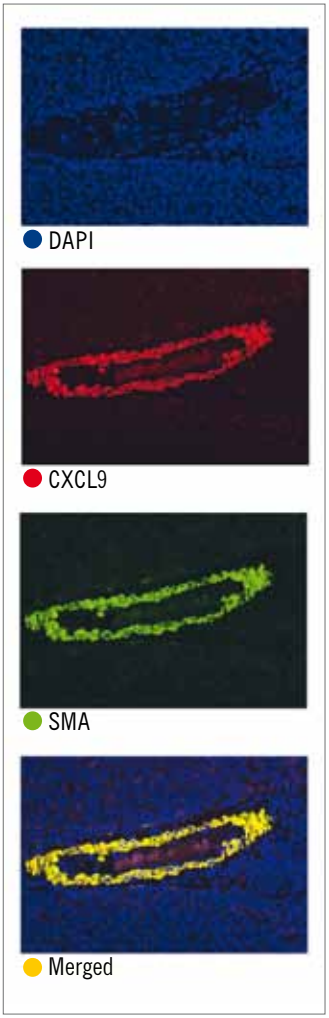
Daniel Venetz, Gabriela Danelon and Mariagrazia Uguccioni

Chronic and persistent inflammation contributes to cancer development and even predisposes to carcinogenesis. Infection-driven inflammations are involved in the pathogenesis of approximately 20% of human tumours. Moreover, even tumours, which are not epidemiologically linked to pathogens, are characterized by the presence of an inflammatory component in the tumour microenvironment. Human cancers possess a complex chemokine network that influences the extent and phenotype of the leukocyte infiltrate, as well as tumour cell and endothelial cell growth and migration. We have expanded our studies on the tumour microenvironment in extranodal lymphomas, focusing on the expression of chemokines that could account for both lymphocyte infiltration, and positioning of malignant B cells in the perivascular cuff. In addition, we have identified additional chemokines that can act in synergism and that are expressed in the perivascular area of primary central nervous system lymphomas. The role of chemokines and molecules that can modulate the activity of chemokine receptor agonists in the tumour microenvironment is at its infancy and is part of our ongoing research.

Primary central nervous system lymphomas (PCNSL) are aggressive malignancies confined to the CNS, mostly of diffuse large B cell histotype. Despite improved understanding of the malignant B cells, little is known about the tumour microenvironment and the response of the adaptive immunity against PCNSL. We investigated the phenotype of tumour infiltrating lymphocytes (TILs), and the expression of chemokines that could affect malignant B cells and trafficking of TILs. TILs and chemokine expression were evaluated by immunohistochemistry and *in situ* hybridization. Furthermore, we performed *in vitro* migration assays to analyze the migratory capacity of lymphocytes and malignant B cells towards chemokines and chemokine heterocomplexes.

In PCNSL from immunocompetent patients, $CD8^+$ T cells represent the majority of TILs in the tumour mass. They tend to accumulate in perivascular areas, express Granzyme B, and proliferate *in situ*. Their localization and density correlates with the expression of the inflammatory chemokine CXCL9, which is transcribed and translated by perivascular macrophages and pericytes in the perivascular microenvironment (Figure 3). Moreover, CXCL9 and CXCL12 are coexpressed on the tumour vasculature and form heterocomplexes. In the presence of CXCL9, CXCL12-induced migration is enhanced not only on $CXCR4^+CXCR3^+CD8^+$ T cells but also on $CXCR4^+CXCR3^-$ malignant B cells. These findings indicate the presence of a strong chemoattractant stimulus in the perivascular microenvironment, which might serve as a regulator for the recruitment of TILs and for the angiocentric positioning of malignant B cells in the perivascular cuff.

» *Venetz D. et al. Int J Cancer 2010, 127:2300-2312.*



↗ ***Figure 3:*** *CXCL9 expression in smooth muscle cells (SMA) in PCNSL endothelium. Double immunofluorescence performed on paraffin-embedded tissues. Magnification 20x.*

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.

Funding

- *European Union*
  INNOCHEM: Innovative Chemokine-based Therapeutic Strategies for Autoimmunity and Chronic Inflammation
  FP6 – LSHP-CT-2005-518167 / 2005-2010
- *European Union*
  DEC-VAC: Development of a Dendritic Cell-targeted Vaccine against AIDS
  FP6 – LSHP-CT-2005-018685 / 2005-2010
- *European Union*
  Marie Curie IEF Fellowship to Valentina Cecchinato
  FP7 - PEOPLE-IEF-2008 / 2009-2011
- *Swiss National Science Foundation*
  Impact of multiple chemokine expression in human disease
  3100A0- 118048-1 / 2008-2011
- *San Salvatore Foundation*
  Chemokine expression in extranodal lymphomas
  2008-2011

Collaborations

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  William Harvey Institute, London (UK)
- *Ugo D'oro*
  Novartis, Siena (IT)

Visiting Scientists

- *Noemi D'Atanasio*
  A.C.R.A.F. S.p.A, Rome (IT)

Publications

- ***Perivascular expression of CXCL9 and CXCL12 in primary central nervous system lymphoma: T-cell infiltration and positioning of malignant B cells.***
  Venetz, D., M. Ponzoni, M. Schiraldi, A. J. Ferreri, F. Bertoni, C. Doglioni, and M. Uguccioni. Int J Cancer. 2010; 127:2300-2312.

Lectures and Seminars

- *IOSI*
  Seminar "Chemokine activities in haematological disorders"
  Bellinzona (CH) / 13.01.2010
- *A.C.R.A.F. S.p.A.*
  Seminar" Chemokines in chronic inflammation"
  Rome (IT) / 17.02.2010
- *Gordon Research Conference on Chemotactic Cytokines*
  Chemokines in extranodal lymphomas
  Il Ciocco (IT) / 30.05.2010
- *INNOCHEM meeting*
  Synergy-inducing chemokines
  Berlin (DE) / 11.10.2010
- *EADV meeting*
  Chemokines in skin diseases
  Bellinzona (CH) / 18.11.2010

Luca Varani
Structural Biology

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


WATER
Milli Q Direct

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

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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).

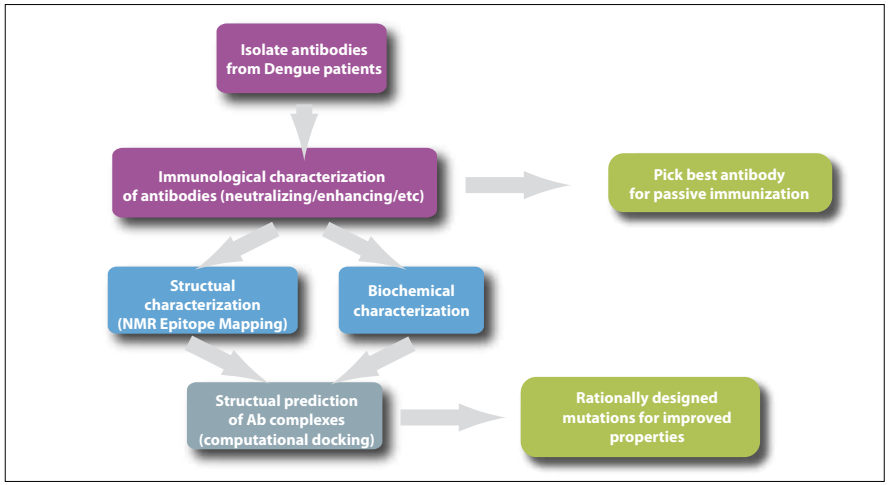


Figure 1 *Experimental workflow*

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.

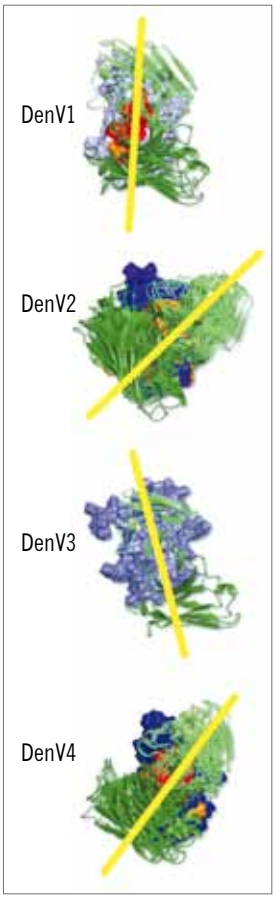
» *Simonelli L. et al. J Mol Biol 2010, 5: 1491-507.*

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.

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 domainIII 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.

Details from July 2007 until June 2009

Funding

- *SVRI*
  Computer assisted vaccine design
  2009-2010
- *CSCS*
  Prediction and characterization of intermolecular interactions in human diseases
  2007-2010
- *European Union*
  EU-NMR – NMR Epitope Mapping: experimentally validated computational biology
  2010
- *MRC Biomedical NMR Centre*
  Characterization of antibody-protein interaction in Dengue virus
  2010

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

- ***The Diversity of Nuclear Magnetic Resonance Spectroscopy.***
  Liu, C. W., V.Y. Alekseyev, J. R. Allwardt, A. J. Bankovich, B. J. Cade-Menun, R. W. Davis, L. S. Du, K. C. Garcia, D. Herschlag, C. Khosla, D. A. Kraut, Q. Li, B. Null, J. D. Puglisi, P. A. Sigala, J. F. Stebbins, and L. Varani.
  Biophysics and the Challenges of Emerging Threats. 2009; 65-81.
- ***Rapid structural characterization of human antibody-antigen complexes through experimentally validated computational docking.***
  Simonelli, L., M. Beltramello, Z. Yudina, A. Macagno, L. Calzolai, and L. Varani.
  J Mol Biol. 2010; 396:1491-1507.
- ***The human immune response to Dengue virus is dominated by highly cross-reactive antibodies endowed with neutralizing and enhancing activity.***
  Beltramello, M., K. L. Williams, C. P. Simmons, A. Macagno, L. Simonelli, N. T. Quyen, S. Sukupolvi-Petty, E. Navarro-Sanchez, P. R. Young, A. M. de Silva, F. A. Rey, L. Varani, S. S. Whitehead, M. S. Diamond, E. Harris, A. Lanzavecchia, and F. Sallusto.
  Cell Host Microbe. 2010; 8:271-283.
- ***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.

Lectures and Seminars

- *Swiss Clinical Chemistry Congress*
  Lugano (CH) / 09.2009
- *Nanobiotechnology International Workshop: protein-surface interactions*
  Ispra (IT) / 12.2009
- *World-Wide Magnetic Resonance Conference and GIDRM*
  Firenze (IT) / 07.2010
- *Goethe University of Frankfurt*
  Frankfurt (DE) / 07.2010
- *MipTec 2010*
  Basel (CH) / 09.2010

SECTION 2

PhD PROGRAMME

INDEX SECTION 2 – PhD PROGRAMME

Andrea Reboldi

Constitutive and inflammatory pathways of T cell trafficking into the CNS during experimental autoimmune encephalomyelitis

Supervisor: Federica Sallusto // Co-referee: Dirk Busch

PhD Program in Molecular Medicine, Vita Salute San Raffaele University, Milan, Italy

Different types of $CD4^+$ T helper (Th) cells develop from naive T cells under the influence of polarizing signals and master transcription factors. Th1 cells require IL-12, IFN-γ and the transcription factor T-bet and, through production of IFN-γ and activation of macrophages, mediate protection against intracellular pathogens such as Mycobacterium tuberculosis. Th2 cells require IL-4 and the transcription factor GATA-3 and, through production of IL-4, IL-5 and IL-13, mediate protection against extracellular parasites. A recently identified lineage of effector $CD4^+$ Th lymphocytes produces IL-17, a cytokine that induces production of chemokines and antimicrobial peptides by tissue cells leading to recruitment of neutrophils and inflammation. Mouse Th17 cell differentiation requires TGF-γ and IL-6 and the transcription factor RORγt. Besides their possible role in host defence against microbes, Th17 cells have been implicated in the pathogenesis of several inflammatory and autoimmune diseases. Th17 play a key role in induction of experimental autoimmune encephalomyelitis (EAE), the mouse model of multiple sclerosis. However, the mechanisms by which Th17 cells migrate into the brain and elicit EAE remained to be determined. The aim of the project was to define the trafficking properties of Th17 cells, in particular to assess the role of CCR6, a chemokine receptor preferentially expressed on human and mouse Th17 cells, in Th17-mediated immune responses. We found that mice lacking CCR6, a chemokine receptor characteristic of Th17 cells, developed Th17 responses, but were highly resistant to induction of EAE. Disease susceptibility was reconstituted by transfer of wild-type T cells that entered into the central nervous system (CNS) before disease onset, and triggered a massive CCR6-independent recruitment of effector T cells across activated parenchymal vessels. The CCR6 ligand CCL20 was constitutively expressed in epithelial cells of choroid plexus in mice and humans. These results reveal distinct molecular requirements and ports of lymphocyte entry into non-inflamed versus inflamed CNS and suggest that the CCR6-CCL20 axis in the choroid plexus controls immune surveillance of the CNS.

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

» *Grégoire C. et al. Eur J Immunol. 2008, 38:2076-2084.*

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

Dirk Baumjohann

Dissection of the reciprocal interaction between T helper cells and B cells during immune responses in vivo

Supervisor: Federica Sallusto // Co-referees: Christoph Müller and Thomas Brunner
Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland

Cognate interaction between T helper cells and B cells is required for the production of high-affinity antibodies and for the generation of memory B cells and long-lived plasma cells. To study the impact of T cell help on humoral immune responses, we transferred low numbers of antigen-specific T cells into T cell-deficient hosts and tracked serum antibody levels, germinal centre reaction, as well as generation of antibody-secreting cells and long-lived plasma cells at different time points after primary and secondary immunization. We found that adoptively transferred antigen-specific $CD4^+$ T cells induced effective primary antibody responses; however, serum antibody levels were not sustained. Further analysis revealed that upon immunization, T cells proliferated extensively and differentiated into dysfunctional T follicular helper (T_{FH}) cells that did not respond to secondary antigenic stimulation and also failed to provide B cell help upon booster immunization. Although immunoglobulin class-switch recombination, initiation of germinal centre reaction, and differentiation of B cells into antibody-secreting, short-lived plasma blasts were normal, affinity-maturation was impaired and long-lived plasma cells were not detectable in the bone marrow. Furthermore, we showed that cognate interaction with B cells was required for the induction of T_{FH} cells and we provided evidence for a previously unappreciated role of dendritic cells (DCs) in the maintenance of functional T_{FH} cells and GCs. The results of this study suggest the existence of distinct early and late T cell-dependent events in the initiation and maintenance of humoral immune responses and of a negative regulatory mechanism by which B cells prevent excessive help from T_{FH} cells during humoral immune responses. Our results have implications not only for the treatment of immunodeficient patients, but also for the understanding of T_{FH} cell function and may translate into the development of more effective vaccination strategies.

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

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

Ulrike Naumann

The function of human CXCR7 (RDC1)

Supervisor: Marcus Thelen // Co-referees: Marlene Wolf and Thomas Brunner

Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland

Cells navigate in the body with the help of chemokines and the respective chemokine receptors in organogenesis, hematopoiesis, homeostasis, and inflammation. The great chemotactic potential of chemokines necessitates a tight regulation at specific anatomical sites in the organism. Upon secretion, chemokine availability can be regulated by seven-transmembrane domain chemokine binding molecules that, as opposed to signaling chemokine receptors, fail to trigger chemotactic responses but instead function as chemokine transporters or scavengers. These 'silent' chemokine receptors are known as interceptors or decoys.

CXCL12 (SDF-1, stromal cell-derived factor 1) is a chemokine with multiple functions in development and leukocyte trafficking. Disruption of either CXCL12 or its signaling receptor, CXCR4, produces similar neuronal, hematopoietic and cardiac defects in mice, which lead to perinatal death. CXCR7, also known as CMKOR1 or RDC1, was recently established as the second CXCL12 receptor, which also binds CXCL11. CXCR7 does not appear to couple to G proteins or other conventional chemokine receptor signaling pathways.

Opposing views on the function of CXCR7 have been described. While CXCR7-induced signaling was proposed to occur in human prostate cancer, homing of human renal progenitors, and zebrafish lateral line migration, others reported modulation of CXCR4-mediated effects by CXCR7 via heterodimerization, or ligand sequestration by zebrafish CXCR7.

This study demonstrates the mechanism of action behind CXCR7 function in mammalian cells and tissues, in parallel to CXCR4. The focus lies on intracellular trafficking in the presence and absence of ligand, as well as the effects of CXCR4- versus CXCR7-mediated internalization on the processing of CXCL12. CXCR7-expressing cells are shown to effectively internalize and degrade the ligand with only minor reduction of cell surface levels of the receptor. In unstimulated cells, both human and zebrafish CXCR7 are capable of internalizing and cycling back to the membrane. Functional CXCR7 is expressed at adult mouse heart valves and the human umbilical vein endothelium, where it effectively sequesters fluorescently labeled CXCL12. Thus, CXCR7 appears to be a novel member of the mammalian chemokine scavenger family.

» *Naumann U. et al. PLoS One 2010, 5:e9175.*

Silvia Volpe

Exploring signal events elicited by chemotactic-receptor engagement in migrating cells

Supervisor: Marcus Thelen // Co-referees: Ruggero Pardi and Anne-Catherine Andres
PhD Programs of the University of Bern, Switzerland, and the Vita Salute San Raffaele University, Milan, Italy

Leukocyte migration is characterized by morphological changes, which manifest in a rapid cell polarization downstream of chemotactic receptor activation. Controversial views of the signaling mechanisms include the localization and distribution of chemotactic receptors within the plasma membrane, the importance of receptor internalization for cell migration and the role of PI(3)K activity in cell polarization. The study describes a novel monocytic cell system to investigate the cell migration process. THP-1 cells stably transfected with the α2A-adrenergic receptor (α2AAR) show comparable signal transduction in response to the agonist UK 14'304 as when stimulated with CCL2/MCP-1 acting on the endogenous CCR2. Time-lapse video microscopy using fluorescent protein tagged variants of the receptor reveals a uniform receptor distribution in resting and polarized cells. In this experimental setting, receptor internalization appears to be dispensable for fully motile cells. By taking advantage of the CFP/YFP-based intramolecular FRET reporter in the α2AAR (α2AAR-YFP/CFP) ligand-induced receptor, activation can be observed over the entire plasma membrane. However, PI(3)K activation appears to occur only at the leading edge. Freshly isolated monocytes, in contrast to neutrophils, which are known to maintain their polarization axis, can flip the polar axis. Moving the source of chemoattractant from one end to the other of a polarized cell reveals several differences between monocytes and neutrophils. THP-1 cells can similarly revert their polarization axis. Interestingly, PI(3)K activity, measured with a PIP3 specific fluorescent probe, relocates to the newly formed leading edge. Nevertheless, reversion of the polarization axis is apparently independent of PI(3)K activity as it occurs in the presence of wortmannin, which abrogates PIP3 production.

» *Volpe S. et al. PLoS ONE 2010, 5:e10159.*

Daniel Venetz

Chemokine Microenvironment in Primary Central Nervous System Lymphoma

Supervisor: Mariagrazia Uguccioni // Co-referees: Ed Palmer and Stefan Dirnhofer
MD-PhD Programme, University of Basel, Switzerland

Primary central nervous system lymphomas (PCNSL) are aggressive malignancies confined to the CNS, mostly of diffuse large B cell histotype. Despite improved understanding of the malignant B cells, little is known on the tumour microenvironment and on the response of the adaptive immunity against PCNSL. We investigated the phenotype of tumour infiltrating lymphocytes (TILs), and the expression of chemokines that could affect malignant B cells and trafficking of TILs.

TILs and chemokine expression were evaluated by immunohistochemistry and *in situ* hybridization. Furthermore, we performed *in vitro* migration assays to analyze the migratory capacity of lymphocytes and malignant B cells towards chemokines and synergy-inducing chemokines.

We show in 22 cases of PCNSL from immunocompetent patients that $CD8^+$ T cells represent the majority of TILs in the tumour mass. They tend to accumulate in perivascular areas, express Granzyme B, and proliferate *in situ*. Their localization and density correlates with the expression of the inflammatory chemokine CXCL9, which is transcribed and translated by perivascular macrophages and pericytes in the perivascular microenvironment. Moreover, CXCL9 and CXCL12 are coexpressed on the tumour vasculature and form heterocomplexes. In the presence of CXCL9, CXCL12-induced migration is enhanced not only on $CXCR4^+CXCR3^+CD8^+$ T cells but also on $CXCR4^+CXCR3–$ malignant B cells. These findings indicate the presence of a strong chemoattractant stimulus in the perivascular microenvironment, which might serve as a regulator for the recruitment of TILs and for the angiocentric positioning of malignant B cells in the perivascular cuff.

» *Venetz D. et al. Int J Cancer 2010, 127: 2300-2312.*

Riccardo Bernasconi

Identification and characterization of novel players regulating protein quality control and degradation in the mammalian endoplasmic reticulum

Supervisor: Maurizio Molinari // Co-referees: Markus Aebi and Yves Barral
PhD Programme, ETH-Zurich, Zurich, Switzerland

During the PhD thesis, Riccardo identified and characterized novel factors and defined, at the molecular level, the function of novel pathways regulating glyco-protein quality control and disposal from the mammalian ER. Riccardo showed that bio-physical features, such as the presence or absence of protein-bound oligosaccharides, transmembrane anchors or peptidyl-prolyl bonds in the cis configuration, determine the activation of distinct quality control pathways in the ER lumen that depend on the activity of ER lectins (OS-9.1, OS-9.2 and XTP3-B), luminal enzymes (CyPB), membrane adaptors (SEL1L), and E3 ubiquitin ligases (HRD1and Gp78).

Riccardo was also involved in a study showing that mouse hepatitis virus (MHV), a coronavirus (CoV), exploits the host cell machinery for the COPII-independent vesicular export from the ER of EDEM1, a short-lived regulator of ERAD, to co-opt cellular membranes for replication. We have found that cell infection with MHV causes accumulation of EDEM1 and of at least another short-lived ER chaperone, OS-9, in the virus-induced double membrane vesicles (DMVs) containing the viral replication and transcription complexes (RTCs). DMVs are coated with non-lipidated LC3/Atg8 and LC3/Atg8 downregulation, but not inactivation of the host cell autophagy, protects mammalian cells from CoV infection. This study identifies the host cellular pathway hijacked by CoV, paving the way for the development of new therapies to combat this family of viruses and describes a new autophagy-unrelated role for non-lipidated LC3-I.

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

» *Bernasconi R. et al. Protein misfolding: New Research O'Doherty, C.B. and Byrne, A.C. Eds, Nova Science Publishers, Inc., Hauppauge, NY. 2008, 113-123.*

» *Aebi M. et al. TIBS 2010, 35:74-82.*

» *Bernasconi R. et al. J Cell Biol. 2010, 188:223-235.*

» *Hebert D.N. et al. Semin Cell Dev Biol. 2010, 21:526-532.*

» *Reggiori F. et al. Cell Host & Microbe. 2010, 7:500-508.*

» *Bernasconi R. et al. PLoS ONE 2010, 5:e13008.*

» *Bernasconi R. et al. Curr Opin in Cell Biol. 2011 23:176-183.*

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

Ramon J Mayoral

Pleiotropic effects of microRNA-221-222 in mast cells

Supervisor: Silvia Monticelli; Co-referee: Luigi Naldini

PhD Programme in Molecular Medicine, Vita Salute San Raffaele University, Milan, Italy

Mast cells have essential effector and immunoregulatory functions in IgE-associated allergic disorders and certain innate and adaptive immune responses. To examine the role of two activation-induced microRNAs, miR-221-222, in mast cells, we developed robust lentiviral systems for miRNA overexpression and depletion. Although both miRNAs have the same seed sequence and both potentiated mast cell adherence equivalently, miR-222-overexpressing cells showed a striking increase in mast cell migration to the peritoneal cavity and mesenteric membranes in mice, compared to cells overexpressing miR-221. In contrast, miR-221-overexpressing cells were more effective at degranulating in response to IgE-antigen complexes. Neither miRNA interfered with mast cell differentiation. miR-221 promoted mast cell survival under conditions of SCF withdrawal, and increased mast cell migration towards SCF and antigen in trans-well migration assays. Transcriptional profiling of miR-221-overexpressing cells revealed modulation of many transcripts in mast cells, including several associated with the cytoskeleton. Indeed, miR-221-222 overexpression was associated with reproducible increases in cortical actin. Finally, the transcriptional repressor PLZF potentiated mast cell differentiation, but in a way that seemed unrelated to miR-221-222 expression. Our studies provide new insights into the roles of PLZF and miR-221-222 in mast cell biology, and identify novel mechanisms that may be dysregulated in pathological conditions.

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

» *Mayoral R.J. et al. Methods Mol Biol. 2010, 667:205-14.*

Luca Simonelli

Rapid Structural Characterization of Human Antibody-Antigen Complexes through Experimentally Validated Computational Docking

Supervisor: Luca Varani // Co-referee: Mauro Fasano
PhD Programme, University of Insubria, Varese, Italy

If we understand the structural rules governing antibody (Ab)–antigen (Ag) interactions in 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.

To obtain and compare the three-dimensional structure of a large number of different complexes, however, we need a faster method than traditional experimental techniques. While biocomputational docking is fast, its results might not be accurate. Combining experimental validation with computational prediction may be a solution.

As a proof of concept, we isolated a monoclonal Ab from the blood of a human donor that recovered from dengue virus infection, characterized its immunological properties, and identified its epitope on domain III of the dengue virus E protein through simple and rapid NMR chemical shift mapping experiments. We then obtained the three-dimensional structure of the Ab-Ag complex by computational docking, using the NMR data to drive and validate the results. In an attempt to represent the multiple conformations available to flexible Ab loops, we docked several different starting models and present the result as an ensemble of models equally agreeing with the experimental data. The Ab was shown to bind a region accessible only in part on the viral surface, explaining why it cannot effectively neutralize the virus.

In our study we demonstrated that computational docking is a fast and accurate tool to obtain the structure of antibody-antigen complexes, provided that it is validated by rapidly obtained experimental data. The use of NMR epitope mapping, a fast and reliable technique to identify antibodies epitopes, noticeably improves the docking accuracy.

» *Beltramello M. et al. Cell Host Microbe. 2010, 8:271-283.*

» *Simonelli L. et al. J Mol Biol. 2010, 396:1491-1507.*

» *Pedotti M. et al. Int J Mol Sci. 2011, 12:226-251.*

SECTION 3

PhD LECTURE COURSE & SEMINARS

INDEX SECTION 3 – PhD LECTURE COURSE & SEMINARS

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

PhD Lecture Course 2010

- *Guido Kroemer*
  "Apoptosis and autophagy"
  INSERM, Institut Gustav Roussy, Villejuif, Paris, (FR) / 20.10.2009
- *Ruggero Pardi*
  "Concurrent steps in chemokine-driven transendothelial migration: the role of beta-arrestins"
  Vita-Salute San Raffaele University, School of Medicine, DIBIT-Scientific Institute San Raffaele, Milan, (IT) / 30.10.09
- *Marco Stampanoni*
  "Phase contrast X-ray tomographic microscopy: a new tool in bioimaging"
  Institute for Biomedical Engineering ETH-Zurich and Paul Scherrer Institute, Villigen (CH) / 20.11.09
- *Steffen Jung*
  "Differential Origins and Functions of Intestinal Dendritic Cells"
  The Weizmann Institute of Science (IL) / 21.01.10
- *Stephen J. Galli*
  "The mast cell paradox: Mast cells as positive and negative regulators of innate or adaptive immune responses"
  Stanford University School of Medicine, California (USA) / 03.02.10
- *Hergen Spits*
  "The expanding universe of IL-17 and IL-22-producing lymphocytes"
  University of Amsterdam Center for Immunology Amsterdam (NL) / 04.03.10
- *Reina Mebius*
  "Lymphoid organ development in health and disease"
  VU University Medical Center, Amsterdam (NL) / 14.04.10
- *Andreas Radbruch*
  "Stroma niches and immunological memory"
  German Rheumatism Research Center, Berlin (DE) / 05.05.10
- *David Neuhaus*
  "Two zinc fingers"
  MRC Laboratory of Molecular Biology, Cambridge (UK) / 14.05.10
- *Ineke Braakman*
  "Protein folding at the ER membrane"
  Utrecht University, Utrecht (NL) / 27.05.10
- *Alexander Rudensky*
  "Control of immune homeostasis by regulatory T cells"
  Memorial Sloan-Kettering Institute, New York (USA) / 08.06.10

Seminar Programme 2010

- *Paola Larghi*
  "Role of p50 NF-kappaB in dendritic cell functions"
  Istituto Clinico Humanitas, Milan (IT) / 13.01.2010
- *Quentin Sattentau*
  "HIV and the third synapse"
  Sir William Dunn School of Pathology, University of Oxford (UK) / 22.01.10
- *Davide Ferrari*
  "Selection of specific membrane protein binders to be used in crystallization trials"
  University of Parma (IT) / 12.03.10
- *Natko Nuber*
  "Fine analysis of MAGE-C1/CT7 expression and immunity in melanoma patients"
  University Hospital Zurich (CH) / 28.04.10
- *Ursula Grohmann*
  "Indoleamine 2, 3-dioxygenase and immune regulation: tryptophan starvation and beyond"
  University of Perugia (IT) / 21.04.10
- *Matthias von Herrath*
  "Combination therapies and antigen induced Tregs in type 1 diabetes"
  La Jolla Institute of Allergy & Immunology, California (USA) / 12.05.10
- *Henrik Müller*
  "Characterisation of T cell phenotypes in the immune response to Mycobacterium tuberculosis"
  Max-Planck-Institute for Infection Biology, Berlin (DE) / 21.05.10
- *Paulo Vieira*
  "Signals regulating murine B cell commitment and differentiation"
  Institut Pasteur, Paris (FR) / 28.05.10
- *Romain Marlin*
  "The mucosal innate immunity during pregnancy: Role in the control of HIV-1 transmission?"
  Institut Pasteur, Paris (FR) / 04.06.10
- *Mirco Menigatti*
  "Epigenetic alterations and cancer"
  Institute of Molecular Cancer Research, University of Zurich (CH) / 08.07.10
- *Gabor Gyuelveszi*
  "Not seventeen anymore!"
  University of Zurich (CH) / 16.07.10
- *Leontios Pappas*
  "Plasmodium Vivax: Search for a Vaccine"
  University of Oxford (UK) / 02.08.10
- *Lukas Flatz*
  "Single Cell Gene Expression Profiling Reveals Qualitatively Distinct CD8 T Cells Elicited by Different Gene-Based Vaccines"
  University of Geneva (CH) / 08.07.10
- *Roberto Cattaneo*
  "How receptor-triggered membrane fusion works: the force behind targeting"
  Mayo Clinic College of Medicine, Rochester, New York, (USA) / 09.08.10
- *Cem Gabay*
  "Interleukin-1 modulation in immune and inflammatory responses"
- *University of Geneva (CH) / 03.09.10*
  Susanna Zierler
  "Ion channels in immune function"
  The Queen's Medical Center, Center for Biomedical Research, Honolulu, Hawaii (USA) / 13.09.10
- *Tiziano Tallone*
  "Circulating human monocyte subsets in atherosclerosis and adipose-tissue derived multipotent cells"
  SSCB/Cardiocentro Ticino, Lugano (CH) / 02.11.10
- *Marco Idzko*
  "P2 receptors signaling in inflammatory acute and chronic lung diseases"
  University Hospital Freiburg, Freiburg (DE) / 16.11.10
- *Klaus Seuwen*
  "EBI2 - a G protein coupled receptor sensing novel lipid mediators"
  Novartis Pharma, Basel (CH) / 01.12.2010

SECTION 4

PEOPLE & FINANCES

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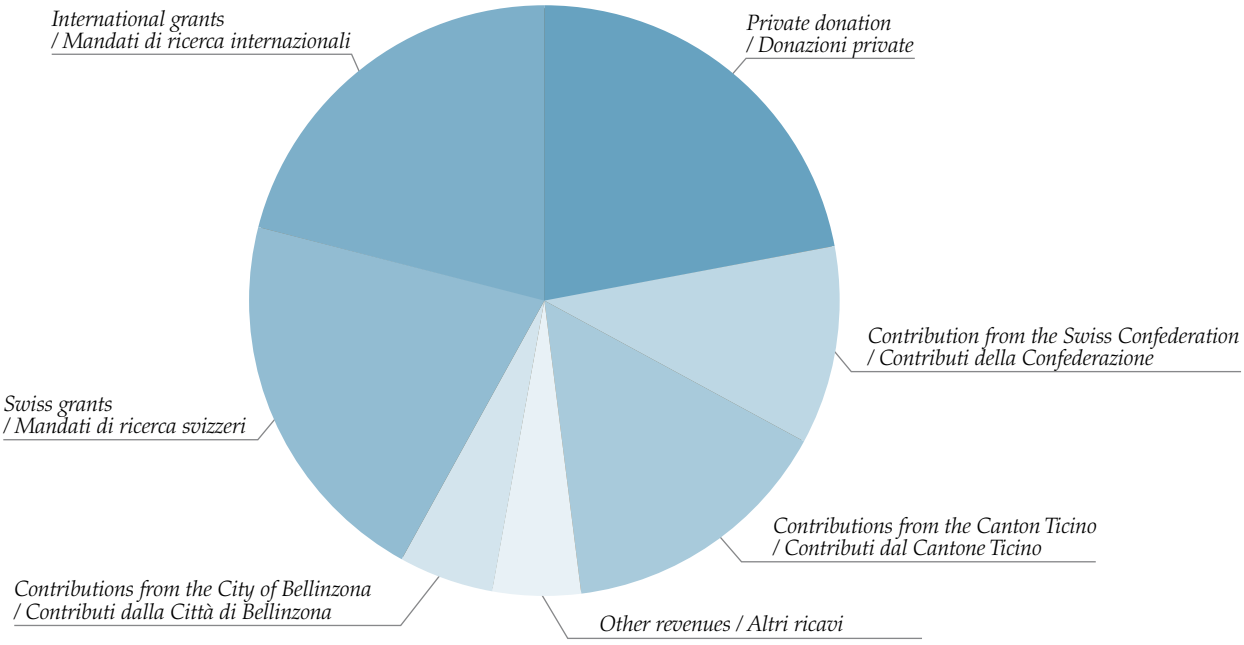
Financial Data 2010 / Dati finanziari 2010

The year 2010 showed a positive result with costs slightly decreased from 13,9 million in 2009 to 13,4 million and revenues at 13,6 million. Funding for research projects (Swiss National Science Foundation, European Union, private Swiss and International Foundations, …) contributed 42% of the overall budget. Half of the cost of research in 2010 was funded by foreign agencies.

L'anno 2010 ha registrato un risultato positivo con costi in leggera diminuzione, scesi da complessivi 13,9 milioni a 13,4 milioni e ricavi pari a 13,6 milioni. I finanziamenti provenienti da progetti di ricerca (Fondo Nazionale Svizzero per la Ricerca, Comunità Europea, Fondazioni provate svizzere e internazionali, …) hanno contribuito a finanziare il 42% del budget dell'istituto. La metà dei finanziamenti della ricerca proviene da agenzie e fondazioni estere.

Funding by source 2010 / Contributi per fonte 2010



Balance Sheet as 31 of December 2010 (In Swiss Francs)
Bilancio al 31 dicembre 2010 (in Franchi Svizzeri)

***ASSETS* / ATTIVO**	**31.12.2010**	**31.12.2009**
1. *Liquidity* / Liquidità	15'866'560	10'169'583
2. *Various Receivables* / Crediti diversi	910'550	912'912
3. *Temporary Receivables* / Transitori attivi	524'584	680'957
Current Assets / Attivo circolante	17'301'694	11'763'452
4. *Participations* / Partecipazioni	12'500	12'500
5. *Buildings* / Immobili	4'841'000	5'143'440
6. *Furnishing & Equipment* / Attrezzature	1'300'000	1'800'000
Fixed Assets / Attivo fisso	6'153'500	6'955'940
Total Assets / Totale attivo	23'455'194	18'719'392

LIABILITIES / PASSIVO	**31.12.2010**	**31.12.2009**
1. *Debt for Delivery and Services* / Debiti per forniture e prestazioni	708'379	701'224
2. *Accruals* / Accantonamenti e transitori passivi	1'742'994	1'781'514
3. *Funds for Research Projects* / Fondi progetti di ricerca	3'886'987	1'716'971
4. *Funds for Laboratories* / Fondi dei laboratori	1'864'259	1'681'807
5. *Various Funds* / Fondi diversi	4'276'765	2'020'875
Current Liabilities /Capitale a breve termine	12'479'384	7'902'391
6. *Long Term Loans* / Prestiti a lungo termine	3'800'000	3'800'000
Long Term Liabilities /Capitale a lungo termine	3'800'000	3'800'000
7. *Capital Resources* / Capitale proprio	7'017'001	6'912'053
8. *Annual Result* / Risultato d'esercizio	158'809	104'948
Equity of the Foundation / Capitale della Fondazione	7'175'810	7'017'001
Total Liabilities / Totale passivo	23'455'194	18'719'392

Profit and Loss Account for the year 2010 (In Swiss Francs)
Conto economico esercizio 2010 (in Franchi svizzeri)

COSTS / COSTI		2010	2009
1.	*Personnel Costs* / Costi del personale	5'736'888	6'256'576
2.	*Consumables* / Fabbisogno medico	1'701'981	2'098'934
3.	*Maintenance of Buildings and Equipment* / Manutenzione immobili e attrezzature	711'466	577'988
4.	*Investments* / Investimenti	1'189'377	817'965
5.	*Amortizations* / Ammortamenti	1'003'826	999'387
6.	*Rent and Related Costs* / Affiti e altri costi dei locali	1'284'692	1'292'616
7.	*Administrative Costs and Various* / Costi generali amministrativi e diversi	935'982	1'030'318
8.	*Travels, Congresses & Guests* / Trasferte, congressi & ospiti	275'792	302'646
9.	*Financial charges* / Oneri finanziari	30'975	32'821
10.	*Various Costs for Research* / Altri costi di ricerca	554'438	463'773
	Total Costs / Totale costi	13'425'417	13'873'024

REVENUES / RICAVI		2010	2009
1.	*Contributions from the Confederation* / *Contributi Confederazione*	1'550'000	1'487'000
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	1'611'667
5.	*Other Contributions / Altri Contributi*	1'187'879	1'229'339
6.	*Research Projects / Progetti di ricerca*	5'700'539	5'966'938
7.	*Other Revenues / Altri ricavi*	657'808	963'028
	Total Revenues / Totale ricavi	13'584'226	13'977'972

ANNUAL RESULT / RISULTATO D'ESERCIZIO	158'809	104'948

SECTION 5

PUBLICATIONS

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

Peer Reviewed Publications
from August 2009

235. Heterogeneity of CD4⁺ memory T cells: functional modules for tailored immunity.
Sallusto, F., and A. Lanzavecchia.
Eur J Immunol. 2009; 39:2076-2082.

236. The Diversity of Nuclear Magnetic Resonance Spectroscopy.
Liu, C. W., V.Y. Alekseyev, J. R. Allwardt, A. J. Bankovich, B. J. Cade-Menun, R. W. Davis, L. S. Du, K. C. Garcia, D. Herschlag, C. Khosla, D. A. Kraut, Q. Li, B. Null, J. D. Puglisi, P. A. Sigala, J. F. Stebbins, and L. Varani.
Biophysics and the Challenges of Emerging Threats. 2009; 65-81.

237. Potent inhibition of HIV-1 by TRIM5-cyclophilin fusion proteins engineered from human components.
Neagu, M.R., P. Ziegler, T. Pertel, C. Strambio-De-Castillia, C. Grutter, G. Martinetti, L. Mazzucchelli, M. Grutter, M.G. Manz, and J. Luban.
J Clin Invest. 2009; 119:3035-3047.

238. Chemokine receptor oligomerization: functional considerations.
Thelen, M., L. M. Munoz, J. M. Rodriguez-Frade, and M. Mellado.
Curr Opin Pharmacol. 2010; 10:38-43.

239. N-glycan structures: recognition and processing in the ER.
Aebi, M., R. Bernasconi, S. Clerc, and M. Molinari.
Trends Biochem Sci. 2010; 35:74-82.

240. Isolation of human monoclonal antibodies that potently neutralize human cytomegalo virus infection by targeting different epitopes on the gH/gL/UL128-131A complex.
Macagno, A., N. L. Bernasconi, F. Vanzetta, E. Dander, A. Sarasini, M. G. Revello, G. Gerna, F. Sallusto, and A. Lanzavecchia.
J Virol. 2010; 84:1005-1013.

241. Hierarchy of immunosuppressive strength among myeloid-derived suppressor cell subsets is determined by GM-CSF.
Dolcetti, L., E. Peranzoni, S. Ugel, I. Marigo, A. Fernandez Gomez, C. Mesa, M. Geilich, G. Winkels, E. Traggiai, A. Casati, F. Grassi, and V. Bronte.
Eur J Immunol. 2010; 40:22-35.

242. Isolation of common dendritic cell progenitors (CDP) from mouse bone marrow.
Onai, N., M.G. Manz, and M.A. Schmid.
Methods Mol Biol. 2010; 595:195-203.

243. ERAD substrates: which way out?
Hebert, D. N., R. Bernasconi, and M. Molinari.
Semin Cell Dev Biol. 2010; 21:526-532.

244. Rapid structural characterization of human antibody-antigen complexes through experi mentally validated computational docking.
Simonelli, L., M. Beltramello, Z. Yudina, A. Macagno, L. Calzolai, and L. Varani.
J Mol Biol. 2010; 396:1491-1507.

245. Analysis of memory B cell responses and isolation of novel monoclonal antibodies with neutralizing breadth from HIV-1-infected individuals.
Corti, D., J. P. Langedijk, A. Hinz, M. S. Seaman, F. Vanzetta, B. M. Fernandez-Rodriguez, C. Silacci, D. Pinna, D. Jarrossay, S. Balla-Jhagjhoorsingh, B. Willems, M. J. Zekveld, H. Dreja, E.
O'Sullivan, C. Pade, C. Orkin, S. A. Jeffs, D. C. Montefiori, D. Davis, W. Weissenhorn, A. McKnight, J. L. Heeney, F. Sallusto, Q. J. Sattentau, R. A. Weiss, and A. Lanzavecchia.
PLoS One. 2010; 5:e8805.

246. Stringent requirement for HRD1, SEL1L, and OS-9/XTP3-B for disposal of ERAD-LS substrates.
Bernasconi, R., C. Galli, V. Calanca, T. Nakajima, and M. Molinari.
J Cell Biol. 2010; 188:223-235.

247. Development of monocytes, macrophages, and dendritic cells.
Geissmann, F., M.G. Manz, S. Jung, M.H. Sieweke, M. Merad, and K. Ley.
Science. 2010. 327:656-661.

248. *Escape from human monoclonal antibody neutralization affects in vitro and in vivo fitness of severe acute respiratory syndrome coronavirus.*
Rockx, B., E. Donaldson, M. Frieman, T. Sheahan, D. Corti, A. Lanzavecchia, and R. S. Baric.
J Infect Dis. 2010; 201:946-955.

249. *CXCR7 functions as a scavenger for CXCL12 and CXCL11.*
Naumann, U., E. Cameroni, M. Pruenster, H. Mahabaleshwar, E. Raz, H. G. Zerwes, A. Rot, and M. Thelen.
PLoS One. 2010; 5:e9175.

250. *CCR6 is expressed on an IL-10-producing, autoreactive memory T cell population with context-dependent regulatory function.*
Rivino, L., P. Gruarin, B. Haringer, S. Steinfelder, L. Lozza, B. Steckel, A. Weick, E. Sugliano, D. Jarrossay, A. A. Kuhl, C. Loddenkemper, S. Abrignani, F. Sallusto, A. Lanzavecchia, and J. Geginat.
J Exp Med. 2010; 207:565-577.

251. *Instructive cytokine signals in dendritic cell lineage commitment.*
Schmid, M.A., D. Kingston, S. Boddupalli, and M.G. Manz.
Immunol Rev. 2010. 234:32-44.

252. *Heterosubtypic neutralizing antibodies are produced by individuals immunized with a seasonal influenza vaccine.*
Corti, D., A. L. Suguitan, Jr., D. Pinna, C. Silacci, B. M. Fernandez-Rodriguez, F. Vanzetta, C. Santos, C. J. Luke, F. J. Torres-Velez, N. J. Temperton, R. A. Weiss, F. Sallusto, K. Subbarao, and A. Lanzavecchia.
J Clin Invest. 2010; 120:1663-1673.

253. *Polarization of migrating monocytic cells is independent of PI 3-kinase activity.*
Volpe, S., S. Thelen, T. Pertel, M. J. Lohse, and M. Thelen.
PLoS One. 2010; 5:e10159.

254. *Human mast cells and mastocytosis: harnessing microRNA expression as a new approach to therapy?*
Deho, L., and S. Monticelli.
Arch Immunol Ther Exp (Warsz). 2010; 58:279-286.

255. *Current Opinion in Immunology. Vaccines. Introduction.*
Ertl, H. C., and A. Lanzavecchia.
Curr Opin Immunol. 2010; 22:355-357.

256. *Coronaviruses Hijack the LC3-I-positive EDEMosomes, ER-derived vesicles exporting short-lived ERAD regulators, for replication.*
Reggiori, F., I. Monastyrska, M. H. Verheije, T. Cali, M. Ulasli, S. Bianchi, R. Bernasconi, C. A. de Haan, and M. Molinari.
Cell Host Microbe. 2010; 7:500-508.

257. *Homeostatic expansion of autoreactive immunoglobulin-secreting cells in the Rag_2 mouse model of Omenn syndrome.*
Cassani, B., P. L. Poliani, V. Marrella, F. Schena, A.V. Sauer, M. Ravanini, D. Strina, C. E. Busse, S. Regenass, H. Wardemann, A. Martini, F. Facchetti, M. van der Burg, A. G. Rolink, P. Vezzoni, F. Grassi, E. Traggiai, and A. Villa.
J Exp Med. 2010; 207:1525-1540.

258. *Selective preservation of bone marrow mature recirculating but not marginal zone B cells in murine models of chronic inflammation.*
Traggiai, E., A. Casati, M. Frascoli, S. Porcellini, M. Ponzoni, F. Sanvito, L. Leng, R. Bucala, L. Moretta, and F. Grassi.
PLoS One. 2010; 5:e11262.

259. *Purinergic control of neutrophil activation.*
Grassi, F.
J Mol Cell Biol. 2010; 2:176-177.

260. *Human anti-HIV-neutralizing antibodies frequently target a conserved epitope essential for viral fitness.*
Pietzsch, J., J. F. Scheid, H. Mouquet, F. Klein, M. S. Seaman, M. Jankovic, D. Corti, A. Lanzavecchia, and M. C. Nussenzweig.
J Exp Med. 2010; 207:1995-2002.

261. *T-cell recognition of chemicals, protein allergens and drugs: towards the development of in vitro assays.*
Martin, S. F., P. R. Esser, S. Schmucker, L. Dietz, D. J. Naisbitt, B.K. Park, M. Vocanson, J. F. Nicolas, M. Keller, W. J. Pichler, M. Peiser, A. Luch, R. Wanner, E. Maggi, A. Cavani, T. Rustemeyer, A. Richter, H. J. Thierse, and F. Sallusto.
Cell Mol Life Sci. 2010; 67:4171-4184.

262. *Autophagy-independent LC3 function in vesicular traffic.*
de Haan, C. A., M. Molinari, and F. Reggiori.
Autophagy. 2010; 6:994-996.

263. *Stable Overexpression of miRNAs in Bone Marrow-Derived Murine Mast Cells Using Lentiviral Expression Vectors.*
Mayoral, R. J., and S. Monticelli.
Methods Mol Biol. 2010; 667:205-214.

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Beltramello, M., K. L. Williams, C. P. Simmons, A. Macagno, L. Simonelli, N. T. Quyen, S. Sukupolvi-Petty, E. Navarro-Sanchez, P. R. Young, A. M. de Silva, F. A. Rey, L. Varani, S. S. Whitehead, M. S. Diamond, E. Harris, A. Lanzavecchia, and F. Sallusto.
Cell Host Microbe. 2010; 8:271-283.

265. *Perivascular expression of CXCL9 and CXCL12 in primary central nervous system lymphoma: T-cell infiltration and positioning of malignant B cells.*
Venetz, D., M. Ponzoni, M. Schiraldi, A. J. Ferreri, F. Bertoni, C. Doglioni, and M. Uguccioni.
Int J Cancer. 2010; 127:2300-2312.

266. *Cyclosporine a-sensitive, cyclophilin B-dependent endoplasmic reticulum-associated degradation.*
Bernasconi, R., T. Solda, C. Galli, T. Pertel, J. Luban, and M. Molinari.
PLoS One. 2010; 5:e13008.

267. *Monocytes join the dendritic cell family.*
Sallusto, F., and A. Lanzavecchia.
Cell. 2010; 143:339-340.

268. *From vaccines to memory and back.*
Sallusto, F., A. Lanzavecchia, K. Araki, and R. Ahmed.
Immunity. 2010; 33:451-463.

269. *Chondroitin Sulfate A-Adhering Plasmodium falciparum-Infected Erythrocytes Express Functionally Important Antibody Epitopes Shared by Multiple Variants.*
Barfod, L., T. Dobrilovic, P. Magistrado, P. Khunrae, F. Viwami, J. Bruun, M. Dahlback, N. L. Bernasconi, M. Fried, D. John, P. E. Duffy, A. Salanti, A. Lanzavecchia, C. T. Lim, N. T. Ndam, M. K. Higgins, and L. Hviid.
J Immunol. 2010; 185:7553-7561.

270. *CD4(+) CD25(+) Treg regulate the contribution of CD8(+) T-cell subsets in repopulation of the lymphopenic environment.*
Almeida, A. R., I. F. Ciernik, F. Sallusto, and A. Lanzavecchia.
Eur J Immunol. 2010; 40:3478-3488.

271. *Crystal Structure and Size-Dependent Neutralization Properties of HK20, a Human Monoclonal Antibody Binding to the Highly Conserved Heptad Repeat 1 of gp41.*
Sabin, C., D. Corti, V. Buzon, M. S. Seaman, D. Lutje Hulsik, A. Hinz, F. Vanzetta, G. Agatic, C. Silacci, L. Mainetti, G. Scarlatti, F. Sallusto, R. Weiss, A. Lanzavecchia, and W. Weissenhorn.
PLoS Pathog. 2010; 6:e1001195.

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.

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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.

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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.***
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