Università della Svizzera italiana

Institute for Research in Biomedicine

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Scientific Report **2012**

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This Scientific Report covers the 2012 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 and Gabriele Gendotti

Past and Present Presidents of the Foundation Council

The Institute for Research in Biomedicine (IRB), a university-level research institute, has already well affirmed itself worldwide, despite having started its operations only in the year 2000. Its key strengths are a leadership of international fame, a network of excellent collaborations, and an environment that allows for the carrying out of research with limited teaching activity.

2012 was a year rich in events for the IRB. We in fact modified the Foundation Board and elected a new President to make room for new strengths and fresh ideas and to better face the future challenges that we have undertaken for the growth of the IRB. The Presidential transition and the approval of the new Foundation Board of the IRB took place on June 25th, 2012, and were followed by a meeting with the Bellinzona township authorities in which notable scientific results as well as the prospects of expansion of the Institute were presented. The Foundation Board has also finalized a document that describes its future strategies, its mission, its vision, its goals and the predicted evolution of the IRB for the period from 2012 to 2016. To adapt to the continuous evolution of the Institute, both the Rules of the Foundation, examined and approved by the responsible office of the Confederation, and the Rules for the Personnel have been updated.

The main objective of the IRB Foundation is to consolidate the structure of the Institute to achieve a critical mass that can ensure productive stability and better opportunities of generational turnover at the research group level. In this regard, a new group led by Santiago Fernández González has begun its activities on the research of the immune response to infectious agents, and Andrea Cavalli became an associate member of the Institute bringing with him his experience in the development of new methods for combining experimental data with computer simulations, with the objective of obtaining a three-dimensional structure of molecules to better approach functional studies and interactions. All this has been possible thanks to the support of the Mäxi Foundation. Besides these strong points that merit being further enhanced, the Foundation Board has identified, in the framework of the above-mentioned strategic plan for the period 2012-2016, other points that deserve to be taken into consideration for the future progress of the IRB. Among these other points to consider: the creation of centralized research facilities to which all researchers may have access, the implementation of translational research, which does not imply the reduction of basic research that remains a priority of the IRB, and the profiting from intellectual property generated by its own researchers. In future years, there will also be a natural evolution of the teaching and formation. The doctoral school and the possibility of carrying out a Master's thesis at the IRB may also benefit by the development of the project to establish a Master's program in Medicine in Ticino.

In order to resolve logistics problems that the fragmenting into two separate locations has inevitably produced, planning of the construction of a new central building is currently in progress, thanks also to land which was made available to the IRB through the concession of a leasehold estate by the City of Bellinzona. The land on which the new structure could be built would host not only the IRB, but also the laboratories of the Institute of Oncology Research (IOR), the Neurocenter of Southern Switzerland (NSI) and other groups pertaining to the Multisite Hospital of Ticino (EOC).

On behalf of the entire Foundation Board and all the IRB researchers, we renew our gratitude to our principal sponsors: the Helmut Horten Foundation, the Gustav & Ruth Jacob Foundation and the Mäxi Founda-

tion, as well as to the numerous sponsors and private donors that allow the Institute to continue to carry out its research in the best possible way, contributing proactively in transforming the research and knowledge of Ticino and in advancing the development of a more competitive and dynamic economy.

Prof. Dr. med. Giorgio Noseda, *President of the IRB Foundation Board from 1997 to 2012*
Atty. Gabriele Gendotti, *President of the IRB Foundation Board*

Bellinzona, May 2013

Foreword by Antonio Lanzavecchia

Director of the IRB

Below you will find the scientific report of the Institute for Research in Biomedicine (IRB). The report contains a brief description of the research carried out in 2012. The main themes of research focus on the mechanisms of host defence, in particular innate immunity and inflammation, lymphocyte activation and differentiation, defence against viruses and bacteria and protein quality control.

In 2012, the IRB researchers published a total of 29 papers in various scientific journals. In two studies published in the *Journal of Experimental Medicine*, a team led by Mariagrazia Uguccioni and assisted by the groups of Luca Varani and Marcus Thelen identified new interactions between inflammatory proteins and chemokines that modulate cell migration. In a paper published in *Nature*, a team led by Federica Sallusto and Silvia Monticelli identified a molecular switch that determines the inflammatory potential of Th17 cells. These are cells that protect us from some infections, but that can also cause autoimmune diseases. The team led by Fabio Grassi unveiled a critical role that purinergic receptors play in different aspects of the immune response, and Antonio Lanzavecchia and coworkers revealed the role of somatic mutations in the generation of autoantibodies. Finally, Maurizio Molinari revealed new aspects of the cell's response to stress caused by the accumulation of unfolded proteins in the endoplasmic reticulum.

The originality and the relevance of the research performed at the IRB are attested to by the Institute's success in obtaining competitive funding from the Swiss National Science Foundation (SNSF), the European Union and other agencies. Three new projects (ABIRISK, TIMER, TIROTAPS) were funded by the European Union in 2012. In particular, an ERC Advanced grant was awarded to Federica Sallusto for the project PREDICT: "*Dissecting the human T cell response to pathogens, allergens, and self-antigens*". The ERC Advanced Grants are awarded to researchers that have given outstanding contributions in their research fields to allow them to engage in innovative projects. This is the second ERC Advanced Grant that has been awarded to an IRB scientist.

As of November 2012, the IRB now hosts a new research group led by Santiago Fernández González. Santiago, who worked at Harvard Medical School in Boston, will study the initial phases of the immune response to viruses and vaccines using intravital microscopy. This method allows one to visualize, in a living organism, the dynamic interactions between the incoming viruses and the cells of the immune system. Thanks to the support from the Mäxi Foundation and from the R'Equip project of the SNSF, the IRB has purchased a new multiphoton microscope. We are grateful to the Mäxi Foundation for the generous contribution that has made it possible for the IRB to recruit a new group leader, to improve and expand the flow cytometry and microscopy facility, and to create a new core facility for protein production.

The research on protein structure has been strengthened by the recruitment of Andrea Cavalli. As an associate member of the IRB, Andrea will continue his productive collaboration with the Laboratory of Molecular Biology in Cambridge and will start a new research project on protein aggregates in collaboration with Maurizio Molinari. Structural biology, and in particular the study of the antigen-antibody interaction, represent a promising field of scientific collaboration that has led to an effective integration with the Università della Svizzera italiana (USI), to which the IRB has been affiliated since 2010.

The IRB continues to play a significant role in teaching. The PhD program of the IRB has allowed 54 students to obtain their PhD titles from Swiss or European universities. Of these, several have continued their careers with success in the academic world or in the pharmaceutical industry. Thanks to the contribution of the Gustav & Ruth Jacob Foundation, the 26 PhD students that currently work at the IRB can participate in a series of lectures and seminars given by leading experts in their fields.

Over the last few years, the IRB has achieved a better integration into the Swiss academic environment. Besides the affiliation to the USI mentioned above, the IRB also collaborates with the Swiss Federal Institute of Technology (ETHZ), with the University of Zurich, and within the ProDoc program, with the Universities of Bern and Fribourg. Within the framework of a future "Master Medical School Ticino", the IRB could contribute to the teaching of immunology and biology, in particular in the fields of vaccination and of infectious, degenerative and autoimmune diseases.

The IRB can look to the future with optimism. A special thanks goes to Professor Giorgio Noseda who, as President of the Foundation Council, led the IRB through its first 13 years. His leadership, his enthusiasm and his determination have contributed to making Bellinzona a reference point for life sciences south of the Alps. Under his leadership, the IRB has become a core for aggregation by other institutions operating in Ticino in this field, such as the Institute of Oncology Research (IOR), the Cantonal Institute of Microbiology, the Neurocenter of Southern Switzerland and, last but not least, a biotech company, Humabs, which was created as a spin-off company of the IRB. The collaboration agreement between the IRB and Humabs not only guarantees a stream of revenue to the Institute, but also promotes translational research as well as employment in the area.

A final wish goes out to the new President of the Foundation Council, Atty. Gabriele Gendotti, who will accompany the IRB through the coming years, which, on the one hand , will see the realization of the new headquarters in Bellinzona, and on the other, will undergo an effective integration into the USI. Our gratitude also goes out to all the present and past members of the Foundation Council for their success in finding the funds and their energy spent in the commitment to the construction of the new building that will allow the IRB to expand and broaden its areas of research.

The Institute is especially fortunate to receive core funding from its main sponsors, the Helmut Horten Foundation, the City of Bellinzona, the Canton Ticino and the Swiss Confederation. Our gratitude also goes out 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, *Director of the IRB*

Bellinzona, May 2013

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RESEARCH GROUPS
SECTION 1

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Santiago F. González

Infection and Immunity

Santiago F. González

Santiago F. González holds two PhD degrees, one in microbiology from the University of Santiago de Compostela (Spain) and one in immunology from the University of Copenhagen (Denmark). From January 2007 to September 2011 he was a postdoc in the group of Michael Carroll at the Immune Disease Institute, Harvard Medical School, in Boston (USA). He has been awarded two EU Marie Curie Fellowships, one for his postgraduate studies in Denmark where he studied skin inflammation and the connection between innate and adaptive responses from a molecular perspective. The second fellowship was a Marie Curie International Outgoing Fellowship awarded in 2008 for a project shared between Harvard Medical School and the National Center for Biotechnology (Madrid). The project focused on the study of the defense mechanism against Influenza virus. He has published several papers related with antigen trafficking, memory B cell, and the regulation of the immune system in high impact journals. During his work at Harvard he studied the transport mechanism of an influenza vaccine in the lymph node. He found that dendritic cells residing in the lymph node medulla use the lectin receptor SIGN-R1 to capture lymph-borne influenza virus and promote humoral immunity. These results have important implications for the generation of durable humoral immunity to viral pathogens through vaccination and were published in *Nature Immunology*. In November 2012 he joined the Institute for Research in Biomedicine in Bellinzona as a group leader studying pathogen-host interaction.

Research Focus

The primary focus of the laboratory is to study the interface between pathogen and host. The laboratory research interest include the innate and adaptive immune responses to respiratory pathogens, and the mechanisms by which such viruses and bacteria fight the host immune system. The initial response of the body to infection involves a series of events characterized by the rapid up-regulation and recruitment of effectors molecules and cells, which facilitate the elimination of the pathogen and the restoration of homeostasis. However, this response is not unidirectional. The pathogen has developed complex strategies to initially challenge the immune system of the host but also to resist successfully its counter attack. A better understanding of the virulence mechanism of the pathogen will contribute to the development of new strategies directed to fight the infection. In addition, the initial mechanisms in the host response directed to contain the infection will be studied. The combination of the two previous perspectives will contribute to the better understanding of the immune response to the disease challenges, allowing the design of more effective ways to enhance the host immune response.
We are currently using state-or-the-art imaging techniques such as 2-photon intravital microscopy, and confocal microscopy to address some of the aforementioned questions. These techniques enable the study of the interaction between the pathogen and the host in a completely new dimension, monitoring the cell-to-cell and microbe-to-cell interaction in real time. In addition, we will use some classic imaging techniques, such as electron and scanning microscopy, in order increase the resolution and structural information of the infected tissue or cell.

Team

Group Leader: Santiago F. González, PhD, PhD > santiago.gonzalez@irb.usi.ch
Members (from 2013)**:** Yagmur Farsakoglu, PhD student.

Characterization of the inflammatory reaction caused by influenza virus

Yagmur Farsakoglu and Santiago F. Gonzalez

Vaccination against influenza confers in most of the cases protection against the disease. However, the current vaccines are hampered by the need of yearly vaccinations in order to protect against new circulating variants of the virus. Another important limitation of the current influenza vaccines is the development of a suboptimal immunogenicity in the elderly, in patients with chronic diseases, the immunocompromised and in young children, which correlates with higher morbidity and mortality in these risk groups. The aim of this project is to study the relevance of the inflammatory reaction induced by influenza vaccines in the lymph node. We to evaluate the effects that inflammation has on the previously described mechanism of capture and transport of the viral particles from the injection site to the B cell follicle. Understanding the mechanism of action of the influenza vaccine will enable the manipulation of the immune response in order to induce a strongly immunogenic, safer and protective response through vaccination. Moreover, this knowledge is expected to improve other vaccine models, thus benefiting the field of vaccination.

* ***Gonzalez S. et al.***
Nat Immunol. 2010, 11: 427-34.

Role of neutrophils in the immune response to virus and bacteria.

Santiago F. Gonzalez

Despite their abundance and physiological importance not much is known about the role of the neutrophils in the lymph node. Different studies have indicated their important function as major effector cells in controlling infections caused by different types of pathogens. Their mechanism of action is based on the secretion of cytokines and the generation of reactive oxygen species and/or microbiocidal peptides directed towards the pathogen. In addition, some studies have suggested a regulatory role, affecting CD8 T cell priming or interacting with dendritic cells (DC). Interestingly, a recent study has observed a competitive role for the antigen between the neutrophils and the antigen presenting cells (macrophages and DC) in the lymph node. In this work, the authors conclude that neutrophils have an important negative role in the CD4 T cell and B cell responses to three protein antigens: hen egg white lysozyme, ovoalbumin and listeriolysin. In a pilot study we observed that the injection of influenza virus was accompanied with a significant increase of infiltrated neutrophils in the subcapsular sinus area of the drained lymph node. Interestingly, we observed that the infiltrated neutrophils were located in the proximity of the subcapsular sinus macrophages. The aim of this project is to characterize the migration patterns of the infiltrated neutrophils in vivo in the lymph node using different infectious models. In addition, we will evaluate the relevance of this cell type in the initiation of the protective response against different pathogens. To achieve this goal we will use Intravital 2-photon-laser microscopy (Figure).

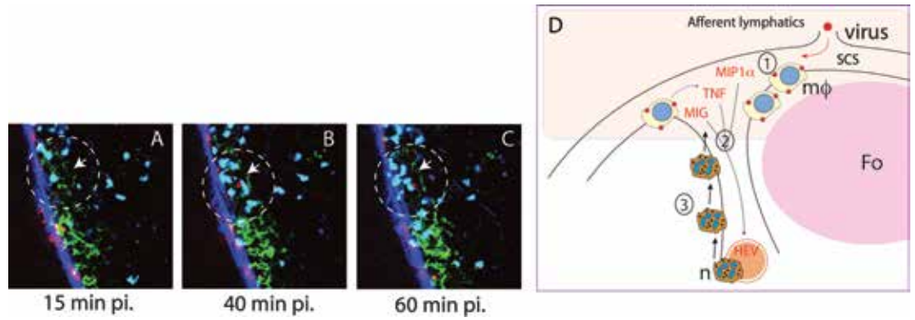

Figure.
(A-C), 2-Photon intravital microscopy snapshots of infiltrated neutrophils (blue) in the subcapsular sinus (SCS) area associated subcapsular sinus macrophages (green). Neutrophils accumulate into the subcapsular sinus macrophage area using a swarming mechanism. (D), Schematic model showing the recruitment of neutrophils in the SCS area.

Funding

Swiss Vaccine Research Institute
Immunity to Flu Vaccine
2012-2014

Swiss National Science Foundation
Acquisition of a 2-Photon microscope
for intravital analysis
R'Equip 316030_145038

Collaborations

Juan Ortin
Centro Nacional de Biotecnología,
Madrid (ES)

Michael Carroll
Harvard Medical School, Boston, MA (US)

Fabio Grassi

T Cell Development

Fabio Grassi

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

Research Focus

Adenosine-triphosphate (ATP) is the source of chemical energy for the majority of cellular functions, serves as a substrate in signal transduction pathways and is incorporated into nucleic acids during DNA replication and transcription. In addition, eukaryotic cells release ATP, which acts 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. Purinergic receptors include non-selective cationic channels (named P2X) and G protein coupled receptors (named P2Y). In the T cell P2X7 is the most abundantly expressed receptor subtype, and has profound impact on T cell responsiveness and metabolism. P2X7 inhibition determines T cell anergy and favours polarization of naïve $CD4^+$ cells toward the immunosuppressive regulatory T cell fate. In contrast prolonged stimulation or high concentration of ATP determine the opening of a pore permeable to molecules up to 900 Da and cell death. P2X7 transcription is developmentally regulated in T cells. We aim at understanding the role of P2X7 in regulating T cell homeostasis and adaptive immunity in different physiological and pathological conditions. We are currently investigating purinergic regulation of T cell metabolism and gut associated lymphoid system as well as mucosal immunity; moreover, we are addressing the role of P2X7 in type 1 diabetes and muscular dystrophy pathophysiology.

Team

Group Leader: Fabio Grassi, MD, PhD > fabio.grassi@irb.usi.ch
Members: Simona Baldassari, PhD student - Vanessa Cornacchione, Diploma student - Caterina Elisa Faliti, PhD student - Lisa Perruzza, PhD student - Michele Proietti, MD, PhD - Tanja Rezzonico Jost, Technician - Rosita Rigoni, Master student - Andrea Romagnani, PhD student.

P2X7 receptor in T cell lineage choice and shaping of γδ cells

Michela Frascoli, Jessica Marcandalli, Ursula Schenk** and Fabio Grassi*
**Former PhD student; **Former Postdoc*

TCR signal strength instructs αβ versus γδ lineage decision in immature T cells. Increased signal strength of γδTCR with respect to pre-TCR results in induction of the γδ differentiation program. Extracellular ATP evokes physiological responses through purinergic P2 receptors expressed in the plasma membrane of virtually all cell types. In peripheral T cells, ATP released upon TCR stimulation enhances MAPK activation through P2X receptors. We investigated whether extracellular ATP and P2X receptors signaling tuned TCR signaling at the αβ/γδ lineage bifurcation checkpoint. We have shown that P2X7 expression is selectively increased in immature $\gamma\delta^{+}CD25^{+}$ cells. These cells are much more competent to release ATP than pre-TCR expressing cells following TCR stimulation and Ca^{2+} influx. Genetic ablation as well as pharmacological antagonism of P2X7 resulted in impaired ERK phosphorylation (Figure 1), reduction of early growth response (Egr) transcripts induction, and diversion of γδTCR-expressing thymocytes toward the αβ lineage fate. The impairment of the ERK-Egr-inhibitor of differentiation 3 (Id3) signaling pathway in γδ cells from *p2rx7*$^{-/-}$ mice resulted in increased representation of the Id3-independent NK1.1-expressing γδ T cell subset in the periphery. Our results indicate that ATP release and P2X7 signaling upon γδTCR expression in immature thymocytes constitutes an important costimulus in T cell lineage choice through the ERK-Egr-Id3 signaling pathway and contributes to shaping the peripheral γδ T cell compartment.

*** *Frascoli M. et al.***
The Journal of Immunology. 2012, 189: 174–180.

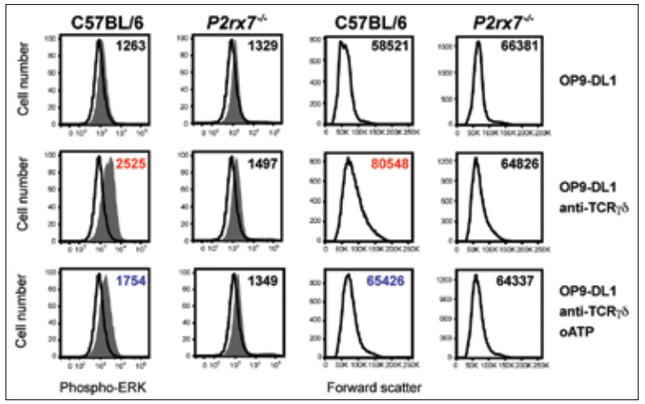

Figure 1. Role of P2X7 in determining γδTCR signal strength during T cell development in the thymus. *Analysis in flow cytometry of ERK phosphorylation and forward scatter in $\gamma\delta^{+}CD25^{+}$ immature thymocytes cocultured with OP9-DL1 stromal cells either untreated or treated as indicated. Sixty-two percent and 70% inhibitions of ERK phosphorylation and cell size increase, respectively, were observed by treatment with P2X antagonist periodate oxidized ATP (oATP) in WT cells stimulated with anti-γδTCR (10 μg/ml) mAb. No variations in ERK phosphorylation and cell size increase were observed in cells with deletion of p2rx7 upon anti-TCR stimulation.*

Regulation of mucosal immunity in the gut by purinergic P2X7 receptor

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

Host's physiology and diet influence the initial development of gut ecosystem in mammals. Gut microbiota in turn promotes the development of the gut associated lymphoid tissue (GALT), which comprises Peyer's patches (PPs), mesenteric lymph nodes (MLNs) and isolated lymphoid follicles. Several layers of control, including mucus, antimicrobial peptides, innate and adaptive immune system ensure a healthy homeostatic relationship of the host with symbionts. Whether bacterial metabolites can regulate host response to ensure commensalism is unknown. We have shown that the *p2rx7* gene is selectively expressed in follicular T helper (T_{FH}) cells in PPs of the small intestine. T_{FH} cells are exquisitely sensitive to P2X7-mediated apoptosis by endoluminal ATP. Deletion of *p2rx7* resulted in expansion of T_{FH} cells and enhanced germinal center reaction in PPs with consequent increase of secretory IgA. In contrast, administration of bactericidal antibiotics and ATP release from bacteria provoked massive P2X7 dependent cell death of T_{FH} cells. Expansion of T_{FH} cells and increase in IgA by deletion of *p2rx7* significantly modified gut microflora abundance and composition. Therefore, ATP is a bacterial metabolite, which regulates gut commensalism by purging follicular help to B cells and IgA response (Figure 2).

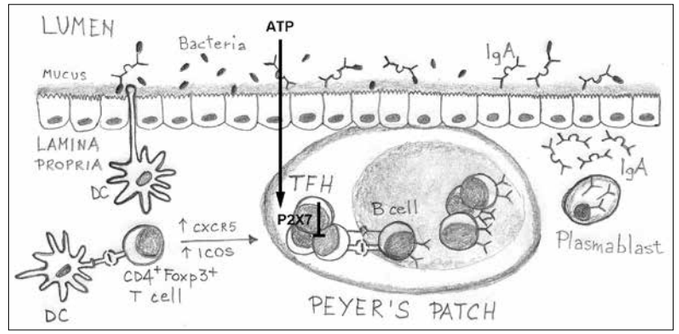

Figure 2. Regulation of follicular helper T cells in Peyer's patches by endoluminal ATP and P2X7 signaling. *The cartoon shows a Peyer's patch in the small intestine with follicular helper T cells (T_{FH}) expressing P2X7. Endoluminal ATP provokes cell death of T_{FH} cells by P2X7 stimulation, thereby regulating plasma cells differentiation and mucosal IgA secretion.*

Purinergic P2X7 receptor in type 1 diabetes

Lisa Perruzza and Fabio Grassi

Although *p2rx7* has been proposed as a type 1 diabetes (T1D) susceptibility gene in non-obese diabetic (NOD) mice, its potential pathogenic role has not been directly determined. To test this possibility we investigated *p2rx7* expression in various T cells subsets namely $CD4^{+}CD62L^{high}CD44^{low}$ naive and $CD4^{+}CD62L^{low}CD44^{high}$ effector from pancreatic lymph nodes of healthy, prediabetic and overtly diabetic NOD mice. *p2rx7* expression significantly increases in $CD4^{+}$ T effector cells of prediabetic NOD mice but dramatically decreases in NOD mice with overt disease. Since *p2rx7* is silenced by cognate antigen stimulation these observations underscore the relevance of pancreatic epitope spreading in the development of T1D in NOD mice. Downregulation of *p2rx7* would render effector T cells resistant to apoptosis induction by extracellular ATP generated by inflammatory tissue damage, thereby propagating and sustaining tissue

destruction. The role of P2X7 activity in limiting the T cell diabetogenic potential was supported by T1D induction with low-dose of streptozotocin in *p2rx7* knock-out mice, which developed a significantly more severe disease than the wild-type counterpart. Our study suggests that P2X7 could constitute a therapeutic target in the early phases of T1D by promoting apoptosis of potentially diabetogenic effector T cells.

Purinergic antagonism in muscular dystrophy

Simona Baldassari and Fabio Grassi

The primary cause of Duchenne muscular dystrophy (DMD) is due to mutations in the dystrophin gene, which encodes a 427 kDa protein (dystrophin) found throughout the cytoplasmic face of the plasma membrane in both skeletal and cardiac muscle. Dystrophin binds distinct members of the dystrophin glycoprotein complex (DCG), forming a 'mechanical-signaling' link from the extracellular matrix to the cytoskeleton. Mutations in dystrophin result in a mechanically weaker plasma membrane, which is more easily damaged during muscle contraction, allowing massive infiltration of immune cells, chronic inflammation, necrosis, and muscle degeneration. Therefore, although dystrophin mutations represent the primary cause of DMD, the secondary processes involving persistent inflammation and impaired regeneration aggravate the disease progression. Administration of periodate–oxidized ATP (oATP), a potent irreversible antagonist of the P2X receptors, to dystrophin-deficient *mdx* mice delayed the progression of the dystrophic phenotype. We observed a significant decrease in the number of muscle tissue CD3 cells. However, the level of Foxp3 transcripts was significantly increased in oATP treated *mdx* mice with respect to *mdx* mice injected with PBS as well as to wild-type mice suggesting the induction of immunosuppressive regulatory T cells in skeletal muscle upon pharmacological P2X antagonism.

Ectonucleotidase activity in class switch recombination of murine B cells

Caterina Elisa Faliti and Fabio Grassi

Class switch recombination (CSR) is an irreversible somatic recombination mechanism by which B cells switch their surface immunoglobulin class expression from IgM and IgD to other isotype with distinct effector function. This diversification is essential for a protective adaptive humoral immune response. B cell receptor (BCR) activation together with exogenous signals, including tumor necrosis factor (TNF) family members, Toll-like receptors (TLRs) ligands or cytokines trigger CSR. We have shown that extracellular adenosine critically contributes to CSR in murine mature B cells. Murine naïve B cells release ATP upon coordinate B cell receptor (BCR) and Toll Like Receptors (TLRs) stimulation. The released ATP is hydrolysed to adenosine by plasma membrane ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1) CD39 and ecto-5'-nucleotidase (5'-NT) CD73. Remarkably germinal center and isotype switched B cells display higher expression of CD73. Moreover, CD39 and CD73 deficient B cells showed in vitro a significant impairment in their capacity to differentiate to class switch plasma cells upon BCR and TLR engagement, underlying the role of adenosine in the B cells isotype switching process. Ig CSR-deficiencies in human can be dependent from an intrinsic B cell defect, however most of them are still molecularly undefined and diagnosed as common variable immunodeficiency (CVID). Notably, CVID patients with impaired class switched antibody responses are selectively deficient in CD73 expression in B cells, suggesting that CD73 dependent adenosine generation contributes to the pathogenesis of this disease.

Induced thymus development as a therapeutic approach in Omenn Syndrome

Anna Casati and Fabio Grassi*
**Former PhD student*

Marrella V. et al.*
Blood. 2012, 120:1005-1014.

Omenn syndrome (OS) is an atypical primary immunodeficiency characterized by severe autoimmunity because of activated T cells infiltrating target organs. The impaired recombinase activity in OS severely affects expression of the pre-T-cell receptor (pre-TCR) complex in immature thymocytes. The pre-TCR promotes αβ T cell development and fosters differentiation of the thymic epithelial component. We have previously shown that anti-CD3ε monoclonal antibody (mAb) treatment in RAG2$^{-/-}$ mice mimics pre-TCR signaling and promotes thymic expansion. We addressed the effect of anti-CD3ε mAb administration in the RAG2^{R229Q} mouse model, which closely recapitulates human OS. These animals, in spite of the inability to induce the autoimmune regulator, displayed a significant amelioration in thymic epithelial compartment and an important reduction of peripheral T-cell activation and tissue infiltration. Furthermore, by injecting a high number of *RAG2*^{R229Q} progenitors into *RAG2*$^{-/-}$ animals previously conditioned with anti-CD3ε mAb, we detected autoimmune regulator expression together with the absence of peripheral immunopathology. These observations indicate that improving epithelial thymic function might ameliorate the detrimental behavior of the cell-autonomous RAG defect. Our data provide therapeutic proof of concept for future clinical applications of anti-CD3ε mAb treatment in severe combined immunodeficiency forms characterized by poor thymus function and autoimmunity (Figure 3).

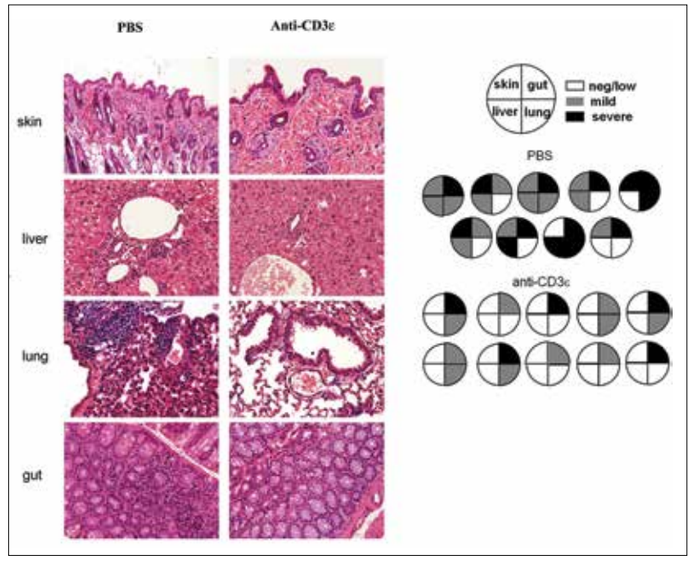

Figure 3. Prevention of immunopathology in RAG2^{R229Q} mice by neonatal administration of anti-CD3ε mAb. *Left, representative hematoxilin and eosin (H&E) staining of skin, liver, lung and gut from PBS and anti-CD3ε treated mice. Right, pie charts show global infiltration grade in each organ calculated from H&E staining. Each pie represents a mouse from one of the two groups.*

Development of an implantable system to monitor inflammation and metabolism

Tanja Rezzonico Jost, Michele Proietti and Fabio Grassi

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

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Funding

Swiss National Science Foundation
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310030_124745 / 2012-2015

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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

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TRPM7 in Regulation of T cell subsets and Purinergic Signaling
(Acronym: TIROTAPS)
No. 184 / 2012-2014

Novartis Foundation for Medical Biological Research
Purinergic signalling as possible pharmacological target in type 1 diabetes
12A09 / 2013

Swiss Cancer League
Purinergic signalling in the pathophysiology of central nervous system infiltration in T-ALL
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Identification of novel targets in central nervous system infiltration in acute T lymphoblastic leukaemia
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Role of P2X7 in immune homeostasis of gut mucosa
2013

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Lectures and Seminars

Convegno Associazione Giovani con Diabete
"Tissue specific immunosuppression by regulatory T cells in type 1 diabetes"
Catania (IT) / 26.05.2012

European Academy of Dermatology and Venereology
4th Scientific Meeting "From the benchside to the clinic and beyond"
"Regulation of Ig secretory response in the small intestine by endoluminal ATP"
Bellinzona / 08.11.2012

13th Italian Neuroimmunology Symposium
"Purinergic P2X signaling in T cell physiology"
Sinalunga, Siena (IT) / 31.01.2013

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 worked at the Basel Institute for Immunology and since 1999 he is the Director of the Institute for Research in Biomedicine in Bellinzona. He taught immunology at the Universities of Genova and Siena and since 2009 is Professor of Human Immunology at the Swiss Federal Institute of Technology ETH Zurich. He is Member of the EMBO and Fellow of the Royal College of Physicians. Awarded the EMBO medal in 1988 and the Cloëtta prize in 1999, Antonio Lanzavecchia published more than 250 papers. His research has covered several aspects of immunology: from antigen processing and presentation to dendritic cell biology, from lymphocyte activation and trafficking to T and B cell memory.

Research Focus

The aim of our current work is to unravel the basis of host resistance to infectious diseases to create a new generation of passive antibody therapies and novel vaccines. The human monoclonal antibodies that we isolate from memory B cells and plasma cells can be used not only as drugs for prophylaxis and treatment of infectious diseases, but also as tools to identify vaccine candidates. Besides these translational studies we address fundamental issues with regard to the cellular basis of immunological memory, the role of somatic mutations in the generation of broadly neutralizing antibodies and the relationship between infection and autoimmunity.

Team

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Cross-neutralization of four paramyxoviruses by a human monoclonal antibody

Davide Corti, Andrea Minola, Chiara Silacci, Laurent Perez, Jessica Mercandalli, and Antonio Lanzavecchia

Broadly neutralizing antibodies reactive against several and even all variants of the same viral species have been recently described for influenza and HIV-1. However, whether the breadth of a neutralizing antibody could cross over different viral species remains to be established. Human respiratory syncytial virus (HRSV) and human metapneumovirus (HMPV) are common pathogens that cause severe disease in premature newborns, hospitalized children and immune-compromised patients. We hypothesized that, due to the repeated exposure to these viruses, rare crossneutralizing antibodies may be selected in some individuals. By screening a large number of donors and interrogating more than 100,000 memory B cells, we succeeded in isolating a human monoclonal antibody (MPE8) that potently cross-neutralizes HRSV and HMPV as well as two animal paramyxoviruses, bovine respiratory syncytial virus (BRSV) and pneumonia virus of mice (PVM). In its germline configuration MPE8 is HRSV-specific and its exceptional breadth is achieved by somatic mutations primarily in the light chain variable region. Importantly, MPE8 did not select escape mutants and showed potent prophylactic and therapeutic efficacy not only in animal models of HRSV and HMPV infection, but also in the most relevant model of lethal PVM infection of mice. The core epitope of MPE8 was mapped on a highly conserved beta strand on the pre-fusion F protein, which is not accessible on the post-fusion F protein. Taken together these results indicate that MPE8 targets an epitope that is highly conserved on the prefusion F protein of several paramyxoviruses and suggest that it may be used for prophylaxis and therapy of severe HRSV and HMPV infections in humans.

This work is done in collaboration with Siro Bianchi, Fabrizia Vanzetta, Gloria Agatic and Barbara Guarino, Humabs BioMed, Bellinzona (CH); Federica Sallusto, IRB; Benjamin Marsland, University of Lausanne (CH); Antonio Piralla, Elena Percivalle and Fausto Baldanti, Fondazione IRCCS Policlinico San Matteo, Pavia (IT).

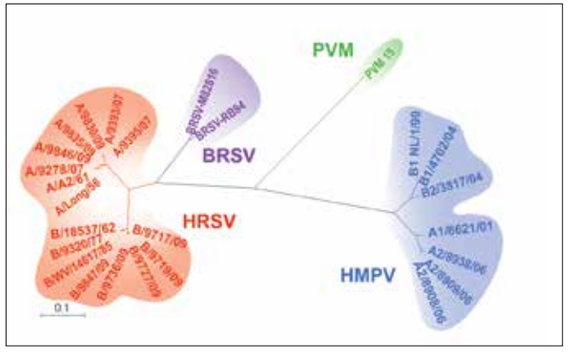

Figure 1.
Dendrogram showing the protein distance of the HRSV, HMPV, BRSV and PVM F proteins.

A stabilized pre-fusion F protein as a candidate vaccine for respiratory syncytial virus

Laurent Perez, Jessica Mercandalli, Chiara Silacci, Davide Corti and Antonio Lanzavecchia

Human respiratory syncytial virus (HRSV) is a common pathogen that causes severe disease in newborns and immune-compromised patients, and plays a major role in acute asthma exacerbations. Surprisingly, in spite of a relative conservation of this virus, there is no evidence that natural infection may lead to long-term protective memory. A neutralizing monoclonal antibody (palivizumab) is used to prevent severe HRSV infection in newborns but, in spite of several attempts, the production of a HRSV vaccine remains a long sought goal. In the search for a broadly neutralizing antibody MPE8, we isolated a large panel of HRSV neutralizing antibodies and characterized 30 of such antibodies in details. All 30 antibodies recognized cells transfected with the HRSV F protein indicating that the F protein is the major target of virus neutralizing antibodies. We then engineered the F protein in its pre-fusion and post-fusion conformations and found that only 3 out of 30 neutralizing antibodies bound to both pre- and post-fusion proteins, while the large majority (27/30 including MPE8 and the most potent neutralizing antibodies) bound exclusively to the pre-fusion protein. The stabilized trimeric pre-fusion and post-fusion F proteins were produced in CHO cells and used to vaccinate mice. The pre-fusion but not the post-fusion vaccine was found to elicit high levels of neutralizing antibodies. Taken together these results identify the pre-fusion F protein as a candidate HRSV vaccine.

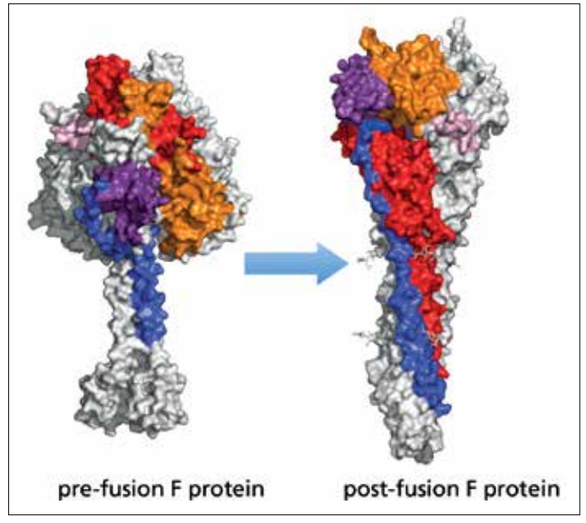

Figure 2.
Conformational change between pre- and post-fusion HRSV F protein.

AncesTree: an algorithm to draw the genealogy tree of clonally related antibodies

Mathilde Perez, Leontios Pappas, Davide Corti and Antonio Lanzavecchia

B cell affinity maturation occurs in germinal centers and is based on the introduction of somatic mutation in immunoglobulin genes followed by selection of the fittest mutants. Analysis of sister clones and the reconstruction of their ancestral relationships allows the reconstruction of genealogical trees illustrating the developmental pathway for the generation of high affinity antibodies and the identification of the key somatic mutations required to achieve high affinity binding. We have developed a novel algorithm to generate genealogy trees from mutated V-gene sequences and their germline unmutated sequences. Original sequences found in experiments are assigned to either terminal or internal nodes of the tree and the branching nodes represents hypotetical developmental clones which are reconstructed by gene synthesis and tested in vitro. An example of a family of antibodies specific for influenza hemagglutinin is shown in figure 3.

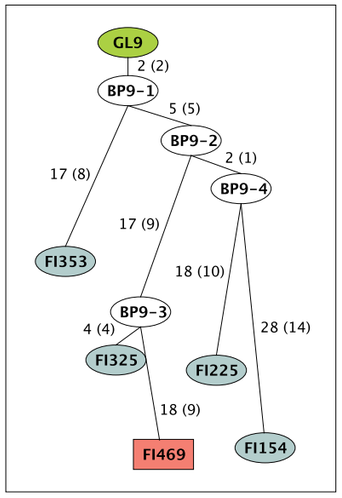

Figure 3.
Genealogy tree of an antibody family reconstructed using AncesTree. Shown are the number of nucleotide mutations and amino acid substitutions (in brackets). Empty symbols indicate hypothetical branchpoints.

The developmental pathway of broadly neutralizing antibodies targeting the stem of the influenza hemagglutinin

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

Understanding the mechanisms that lead to the production of potent and broadly neutralizing antibodies is of both fundamental interest and of practical relevance in the context of vaccine design. We decided to focus our analysis on the neutralizing antibody response to the stem of influenza A hemagglutinin (HA). We have extensively cloned memory B cells and plasma cells from a donor from which serial samples were collected before and after vaccination over a period of 5 years. The sequencing of VH and VL genes allows the precise identification of clonally related B cells. Using the AncesTree algorithm we can draw, for several families, a genealogy tree that identifies early branchpoints as well as highly divergent cells carrying large numbers of somatic mutations. We have sequenced >200 antibodies that had been grouped into 15 families which show distinct dynamics following repetitive antigenic stimulation. We started the analysis form VH1-69 antibodies and confirmed that in these antibodies the specificity for the HA stem is encoded exclusively by the H chain, a fact that facilitates the analysis of the contribution of somatic mutations. By analyzing recombinant antibodies for their binding to HA molecules we observed that the germline antibodies bind poorly to HA stem and that maximal binding is achieved through a limited numbers of somatic mutations which are already present in the first branchpoint, while large numbers of somatic mutations accumulate in terminally differentiated cells without apparent effect on binding. Importantly, and in contrast with published data, we found that the CDR3 region significantly contribute to binding. In addition, we identified a few mutations that increase the affinity of VH1-69 antibodies and found that these mutations may have additive effects. These results suggest that some VH1-69 naïve B cells with permissive CDR3 can bind with low affinity to HA stem and, with only a few mutations can reach high affinity binding. Finally, our analysis provides also some relevant information as to the relationship between memory B cells and plasma cells within the same clone. Interestingly, with a single exception, the antibodies isolated from memory B cells and from plasma blasts differ in the patterns of somatic mutations, suggesting that the two cell types have different selection requirements.

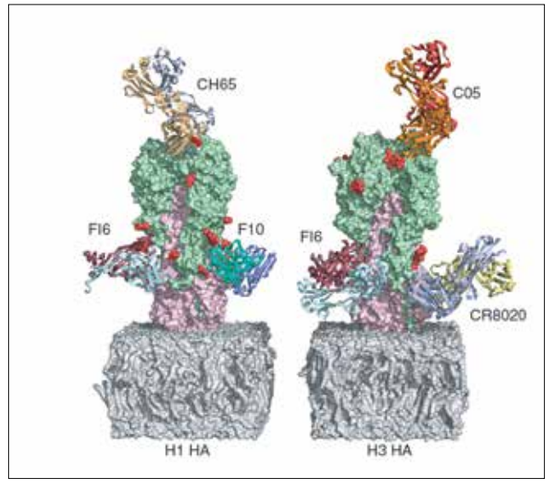

Figure 4.
Model of influenza A H1 and H3 hemagglutinins bound to broadly neutralizing antibodies.

Protecting and pathogenic antibody responses to Dengue virus

Martina Beltramello, Alexander Fruehwirth and Antonio Lanzavecchia

Antibodies can protect against homologous dengue virus (DENV) infection, but can also precipitate severe dengue by promoting heterotypic virus entry via Fcγ receptors (FcγR). We previously reported that human monoclonal antibodies to the domain III (DIII) of the envelope (E) protein potently neutralize DENV infection and are either serotype specific or cross-reactive with two or three but never with all four DENV serotypes. In contrast, DI/DII-specific antibodies have lower neutralizing activity but completely cross-react with the four DENV serotypes. Furthermore we confirmed that all antibodies enhance infection at sub-neutralizing concentrations. The three most potent and broadly neutralizing antibodies were engineered to prevent FcγR binding and found to be devoid of enhancing activity and effective as post-exposure therapy in a mouse model of lethal DENV infection. Based on our findings we hypothesize that the potently neutralizing DIII-specific antibodies protect form homologous but not from heterologous challenge, while the poorly neutralizing DI/II-specific antibodies are the primary culprits for enhancing heterologous infection. We continue the studies on the human antibody response to DENV in collaboration with Federica Sallusto and Luca Varani, IRB and with colleagues based in Ho Chi Minh City, Paris, Berkeley and Chapel Hill.

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

Can serotherapy cure rabies virus infection?

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

Rabies is a lethal disease caused by lyssaviruses belonging to 7 distinct genotypes that is transmitted by the bite of infected mammals and causes ~60.000 deaths per year. The only available treatment is post exposure prophylaxis (PEP) that consists in the administration of low amounts of rabies hyper immune globulins at the site of the bite together with an inactivated rabies genotype 1 vaccine. PEP is effective if administered promptly within 24 hours after the bite but is not effective when the virus has already reached the central nervous system. In addition PEP is less effective for non-genotype 1 lyssaviruses. From donors vaccinated with the genotype 1 vaccine we isolated hundreds of monoclonal antibodies and among these we selected two that potently and broadly neutralize viruses representative all 7 lyssavirus genotypes, including newly isolated eurasian bat viruses. The two antibodies bind to distinct antigenic sites and are therefore suitable candidates for a cocktail that can neutralize most lyssavirus infections including those on which PEP is not effective. In vivo experiments are ongoing in Syrian Hamsters to assess the therapeutic potential of these human monoclonal antibody cocktail.

This work was done in collaboration with Paola De Benedictis and Ilaria Capua, IZV, Legnaro (IT); Herve Bourhy, Institut Pasteur, Paris (FR) and Ed Wright and Robin Weiss, UCL London (UK); Fabrizia Vanzetta and Gloria Agatic, Humabs BioMed SA, Bellinzona (CH).

Immunogenetic mechanisms driving norovirus antigenic variation

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

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

Lindesmith, L. C. et al * *PLoS Pathog. 2012, 8:e1002705.*

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

Long-term culture of normal and malignant plasma cells

Dora Pinto, David Jarrossay and Antonio Lanzavecchia

Plasma cells do not survive in conventional cell cultures a fact that has prevented the in vitro analysis of this cell type. However plasma cells survive in the bone marrow niches organized by mesenchymal stromal cells. 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. We are currently using this method to study myeloma cells in cultures. Our findings indicate that the rate of Ig secretion is lower in myeloma cells as compared to normal plasma cells and that drugs that target autophagy and proteasome function can synergistically inhibit survival of malignant plasma cells.

This work is done in collaboration with Francesca Fontana and Roberto Sitia, HSR, Milano (IT).

A dynamic model of serological memory

Dora Pinto, Leon Pappas, Silvia Preite, Federica Sallusto and Antonio Lanzavecchia

To understand the mechanisms that maintain serological memory we have systematically interrogated the repertoire of recently generate plasma cells ($CD138^+$ DR^+ $CD62L^+$) that are found in peripheral blood. We found that seven days following vaccination with influenza virus most of the recently generated plasma cells produce antibodies specific for the vaccine. However, a sizeable fraction of these recently generated plasma cells produced antibodies of other specificities, including antibodies specific for irrelevant recall antigens. We also found that that in the steady state recently generated plasma cells produced antibodies to vaccines or viruses that the donor had encountered years and even decades before. Representative monoclonal antibodies were isolated from plasma cells to formally demonstrate that in the absence of specific antigenic stimulation there is a continuous generation of plasma cells making memory antibodies. To investigate the mechanism that underpins this polyclonal B cell activation we immunized mice with influenza virus and transferred the memory cells, alone or with memory or naïve T cells, into immunodeficient mice. We found that in the absence of antigenic stimulation serum antibodies to influenza virus slowly but steadily increased over a period of 6 weeks. Interestingly, this increase was observed only in mice transferred with memory B cells and either naïve or memory T cells, but not in mice transferred only with B cells alone. Taken together, these findings support a dynamic model of serological memory where activated T cells drive, in an antigen-independent fashion, the polyclonal stimulation of memory B cells that contributes to maintaining constant levels of plasma cells and consequently lasting levels of protective serum antibodies.

A functional B cell receptor on human IgA and IgM plasma cells

Dora Pinto, Antonio Lanzavecchia and David Jarrossay

Plasma cells are terminally differentiated cells of the B cell lineage that secrete antibodies at high rate and are thought to lack the expression of the B cell receptor (BCR). Clear differences between the IgG and the IgA humoral systems in terms of dynamics have recently emerged. We found that human IgA and IgM unlike IgG plasma cells express a membrane functional BCR associated with the Igα/Igβ heterodimer. BCR crosslinking on IgA and IgM plasma cells led to Ca2+ mobilization, ERK1/2 and AKT phosphorylation and impacted survival of IgA plasma cells. These findings demonstrate fundamentally distinct biology between IgG, IgM and IgA plasma cells and suggest that the IgA plasma cell repertoire may be modulated by the presence of specific antigens.

* ***Pinto, D et al.***
Blood 2013, 16:4110-4114.

This work was done in collaboration with Martin Bolli and Guido Garavaglia, Ospedale San Giovanni, Bellinzona (CH).

Pemphigus autoantibodies are generated through somatic mutations and target the desmoglein-3 cis-interface

Giulia Di Lullo, Davide Corti and Antonio Lanzavecchia*
**Former Master student*

Di Zenzo, G et al.*
J Clin Invest 2012, 122:3781–3790.

Pemphigus vulgaris is an autoimmune blistering disease of skin and mucous membranes caused by autoantibodies to the desmoglein (DSG) family proteins DSG3 and DSG1, leading to loss of keratinocyte cell adhesion. To learn more about pathogenic pemphigus autoantibodies, we isolated 15 IgG antibodies specific for DSG3 from 2 PV patients. Three antibodies disrupted keratinocyte monolayers in vitro, and 2 were pathogenic in a passive transfer model in neonatal mice. The epitopes recognized by the pathogenic antibodies were mapped to the DSG3 extracellular 1 (EC1) and EC2 subdomains, regions involved in cis-adhesive interactions. Using a site-specific serological assay, we found that the cis-adhesive interface on EC1 recognized by the pathogenic antibody PVA224 is the primary target of the autoantibodies present in the serum of PV patients. In contrast, autoantibodies that target the trans-adhesive interface appear to be less frequent in PV patients. The autoantibodies isolated used different heavy- and light-chain variable region genes and carried high levels of somatic mutations in complementary-determining regions, consistent with antigenic selection. Remarkably, binding to DSG3 was lost when somatic mutations were reverted to the germline sequence. These findings identify the cis-adhesive interface of DSG3 as the immunodominant region targeted by pathogenic antibodies in PV and indicate that autoreactivity relies on somatic mutations generated in the response to an antigen unrelated to DSG3.

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

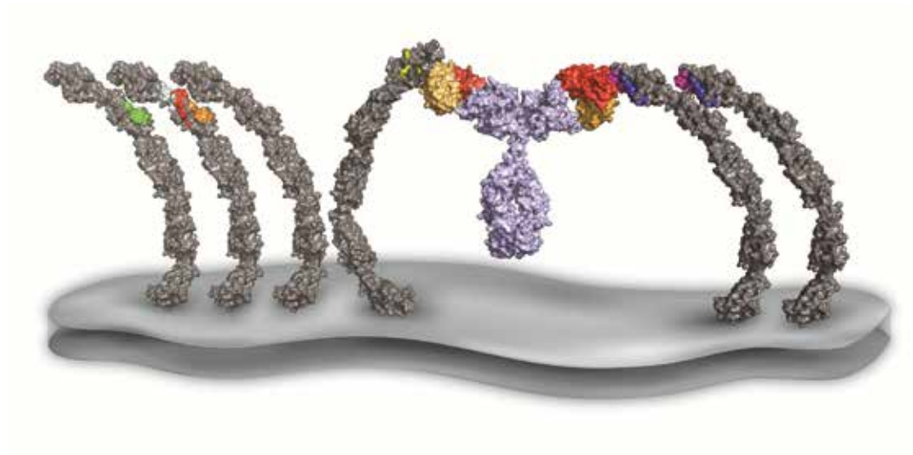

Figure 5.
The study demonstrates how the autoantibodies (in purple) can destroy the net of desmoglein (in grey), which is necessary to maintain the adhesion between the epidermal cells

Autoantibodies to GM-CSF in patients with pulmonary alveolar proteinosis and in healthy individuals: mechanisms of neutralization and role of somatic mutations

Luca Piccoli, Blanca Fernandez-Rodriguez, Laurent Perez, Davide Corti and Antonio Lanzavecchia

Pulmonary alveolar proteinosis (PAP) is a rare severe autoimmune disease caused by autoantibodies that neutralize GM-CSF causing the loss of alveolar macrophages and the accumulation of lipoproteinaceous material within the alveoli. By immortalizing memory B cells from several PAP patients we isolated more that 50 monoclonal antibodies that bind to human GM-CSF with high affinity. Surprisingly, in spite of high neutralizing activity of the patient's serum, none of these antibodies showed GM-CSF neutralizing activity. By cross-competition experiments using surface plasmon resonance we established a map of the antigenic sites of GM-CSF and showed that 3 antibodies can bind simultaneously to a single GM-CSF molecule. Strikingly, while single antibodies were ineffective, a combination of 3 antibodies targeting non-overlapping sites was capable of completely and potently neutralizing the biological activity of GM-CSF. These findings indicate that in PAP the neutralization of GM-CSF is dependent on synergistic antibody combinations. We therefore investigated whether anti-GM-CSF antibodies could be found also in healthy individuals. Using a site-specific serological assays we identified healthy blood donors with low levels on non-neutralizing GM-CSF antibodies. Remarkably, the serum of these donors synergized with some non-neutralizing monoclonal antibodies directed against complementary sites leading to full GM-CSF neutralization. Taken together these results indicate that otherwise healthy individuals can generate autoantibodies to GM-CSF and that in vivo neutralization of GM-CSF observed in PAP patients requires the presence of synergizing antibodies directed against complementary sites.

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

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

Anna Kabanova, Laurent Perez, Daniele Lilleri, Davide Corti and Antonio Lanzavecchia

To understand the cellular tropism of HCMV, identify the molecular targets and characterize the protective T and B cell response, we produced viral glycoprotein complexes in a soluble form. The gH/gL/UL128-131A pentamer complex, the gH/gL dimer and gB were produced in transfected cells, purified and found to preserve all the conformational epitopes targeted by a panel of human neutralizing antibodies. Glycoprotein-specific and site-specific serological assays were developed to monitor the immune response to the virus with the aim of identifying the presence of protective antibodies in serum of pregnant women. The complexes were also used to identify the target molecules on epithelial cells and fibroblasts using co-precipitation and mass spectrometry analysis. The receptor for the gH/gL dimer on fibroblasts was identified and work is ongoing to find the receptor for the gH/gL/UL128-131A pentamer complex on endothelial and epithelial cells using siRNA and candidate gene transfection approaches. Finally, the complexes were tested in mice as candidate vaccines using as adjuvant a non-denaturing polyanionic carbomer. The pentamer was superior to glycoprotein B, which represents a candidate vaccine, both in terms of neutralizing antibody titers and protection of different cellular targets. Through a clonal analysis of the response in vaccinated mice and infected humans, we found that most of the antibodies elicited by the pentamer were neutralizing and most were effective in the picomolar range, while most antibodies elicited by gB were not neutralizing. These results illustrate how potent neutralizing antibodies can be used to identify, within a complex pathogen, the most effective antigens capable of eliciting a protective response, a process that we have defined as analytic vaccinology.

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

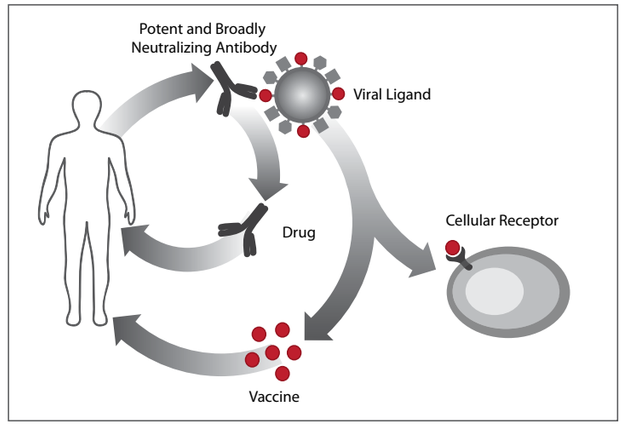

Figure 6.
The use of neutralizing antibodies for serotherapy, vaccine design and as tools for viral receptors discovery

Early maternal antibody response to the gH/gL/pUL pentameric complex correlates with protection from vertical transmission of HCMV

Daniele Lilleri, Anna Kabanova, Davide Corti and Antonio Lanzavecchia

***Lilleri, D. et al.** **
J Clin Immunol. 2012, 32:1324-1331.

***Lilleri, D. et al.** **
PloS One. 2013, 8:e59863.

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

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

Proteomic and metabolomics studies on T cell activation

Roger Geiger and Antonio Lanzavecchia

Naive T cells survive in a resting state and following activation by specific antigen grow, divide and differentiate to acquire different types of effector function. To better understand this process we take an unbiased approach by determining the global changes in proteome and metabolome following T cell activation. Using high-resolution mass spectrometry-based proteomics, we determined the proteome of primary human T lymphocytes in the resting state and after different time points of activation. We identified a total of 6,700 proteins and quantified 4657 of them through label-free quantitation algorithms. Within the first 48 hours after T cell activation the expression of 1005 proteins changed significantly, reflecting the large alterations a cell undergoes. Among those proteins whose expression level increased most, half play a role in metabolism. To study the metabolic changes in more detail we used a mass-spectrometry based metabolomics approach and quantified the changes in 650 metabolites. Integration of the proteomic and metabolomic datasets allowed us to analyze how different metabolic networks respond to the increased energy demands of an activated cell. We are currently probing the identified metabolic pathways directly in T cells by RNA interference or through the use of inhibitors.

This work is done in collaboration with Federica Sallusto, IRB, Nicola Zamboni, ETH Zurich, CH, and Matthias Mann, MPI München (DE).

Identification of cellular factors involved in the generation of memory T cells through shRNA screens

Roger Geiger and Antonio Lanzavecchia

The aim of the project is to use two complementary approaches in physiologically relevant cellular systems to achieve a thorough basic molecular understanding of the human T cell biology and to define principles of regulation of T cell proliferation and differentiation. In the first, genomic approach, we will use an unbiased, genome-wide screening method based on the shRNA technology, to systematically analyze the involvement of cellular factors in the initial events of naïve T cell priming and regulation of cell proliferation. From this functional screening – performed in primary naïve T cells isolated from peripheral blood of healthy adult donors – we will identify genes that positively or negatively regulate cell proliferation and differentiation.

This work is done in collaboration with Federica Sallusto, IRB, Bellinzona (CH).

The human antibody response to gut commensals, pathogens and food antigens

Costanza Casiraghi, Andrea Minola and Antonio Lanzavecchia

Our immune system is continuously exposed to a plethora of antigens derived from commensals, pathogens and food antigens that are supposed to sustain the strong antibody response observed in the gut and other mucosal sites, in particular but not exclusively secretory IgA. However, little is known on the specificity, kinetics and memory of the secretory immune system in humans. Using a variety of approaches we investigate the response to aerobic and anaerobic commensals, pathogens and food antigens in the tissues that are accessible to this analysis. In particular we interrogate memory B cells as well as recently activated B cells and plasma cells that circulate in peripheral blood on their way to mucosal tissues. By determining the frequency of specific cells and characterizing the antibodies produced we expect to gain insight into the normal activity of the immune system and address issues such as the duration of immune responses and immunological memory in the secretory system.

The repertoire of recently activated B cells

Alexander Fruehwirth, Davide Corti and Antonio Lanzavecchia

We are interested to find a method to discriminate recently activated B cells from the bulk of memory B cells. This will offer the possibility of investigating the ongoing activity of the immune system in the steady state and in the course of the response to pathogens. In addition this approach would be extremely valuable to analyse disease activity in autoimmune diseases to identify the factors that elicit and maintain the production of autoantibodies. To identify surface markers of recently activated B cells we used polychromatic flow cytometry and an unbiased analysis program to identify subsets that increase following vaccination or infection. This approach was combined with the immortalization and analysis of the specificity of the specific cells and the isolation of monoclonal antibodies and the analysis of Ki67. Using this approach we were able to identify in peripheral blood a small subset of Ki-67+memory-type B cells that increase 2-4 weeks after vaccination and are highly enriched in vaccine-specific memory cells. After validation of these findings in vaccinated individuals we will start to analyse the repertoire of these cells as compared to bona fide memory cells in healthy individuals and in patients with chronic infections and autoimmune disorders.

The structural basis of potent and broadly H5N1 influenza virus neutralization

Davide Corti, Blanca Fernandez-Rodriguez and Antonio Lanzavecchia

High-affinity neutralizing antibodies against conserved epitopes on the HA globular head could provide immunity to diverse influenza H5N1 virus strains and protection against future pandemic viruses. We have previously isolated from patients that survived avian influenza infection a large panel of human monoclonal antibodies that neutralize H5N1 viruses. One of these antibodies, FLD194, exhibited potent neutralization activity against all H5 clades and subclades tested, including the transmissible reassortant H5 strain. FLD194 showed potent prophylactic and therapeutic efficacy against highly pathogenic avian influenza H5N1 viruses in mice and ferrets. The identification of natural escape variants identified a key mutation at the conserved Q119. FLD194 was co-crystallized with H5 A/VietNam/1203/04 HA and structural data reveal the basis for its potent and broad neutralizing activity.

This work is done in collaboration with Alex Xiong and John Skehel, NIMR, London (UK) and Isabella Monne and Ilaria Capua, IZV, Legnaro (IT).

The role of somatic mutations in the generation of broadly neutralizing antibodies and autoantibodies

Davide Corti and Antonio Lanzavecchia

The mechanism of somatic mutation is responsible for the diversification of the antibodies in germinal centers leading to affinity maturation through the generation of variants that are selected for their fitness for the antigen. Our recent studies on influenza- and HRSV/HMPV-neutralizing have clearly shown that somatic mutations offer also the possibility of broadening the antibody specificity thus anticipating viral evolution. While somatic mutation can provide useful mechanisms such as affinity maturation and repertoire broadening it also poses some risks such as the somatic generation of antibodies that react against self-antigens. Based on our recent results obtained in the study autoantibodies found in pemphigus, PAP and rheumatoid arthritis patients and on a previous report on a lupus autoantibodies (Wellmann, PNAS 2005), we suggest that these newly generated autoreactive B cells that fail to be tolerized in germinal centers may enter the memory pool and be stimulated by self antigens found in peripheral tissues. Environmental and genetic factors may contribute to the expansion and differentiation of these autoreactive B cells. These factors may range from the release of self-antigens following necrotic or infectious events to genetic polymorphisms that facilitate the activation of memory B cells.

Prevention of mucosal SHIV transmission IgA1, but not IgA2, neutralizing monoclonal antibody correlates with differential virion capture and inhibition of transcytosis

Davide Corti and Antonio Lanzavecchia

Passive immunization with anti-HIV-1 IgG1 neutralizing monoclonal antibodies prevents SHIV infection in rhesus monkeys. The role of IgA neutralizing antibodies in preventing mucosal SHIV infection has not been established. IgA1, IgA2, and IgG1 versions of the anti-V3 neutralizing antibody HGN194 were applied intrarectally in rhesus monkeys before intrarectal SHIV challenge. In spite of similar neutralizing activity of the three HGN194 variants, IgA1 provided the best protection in vivo. Five out of the six IgA1-treated rhesus monkeys remained virus-free compared to only one out of six animals receiving IgA2 and two out of six rhesus monkeys treated with IgG1 HGN194. Protection correlated significantly with virion capture activity, as well as inhibition of transcytosis of cell-free virus across an epithelial cell layer in vitro. These data imply that IgA1-mediated capturing of virions in mucosal secretions and inhibition of transcytosis can provide significant prevention of lentiviral acquisition over and above direct virus neutralization. Vaccine strategies capable of eliciying mucosal IgA, especially IgA1, should be therefore developed as a first line of defense against HIV-1.

This work is done in collaboration with Jennifer Watkins and Ruth Rupreht, Dana-Farber Cancer Institute, Boston, MA (US).

The human immune response to HBV

Elisabetta Loggi, Davide Corti and Antonio Lanzavecchia

Chronic infection by hepatitis B virus (HBV) remains a major health problem with 350 million chronic carriers worldwide of which 15-40% develop liver cirrhosis and hepatocellular carcinoma. Chronic patients are currently treated with polymerase inhibitors or IFN-α, but the rate of seroconversion is very low. Hepatitis B immune globulins (HBIG) are used for post-exposure prophylaxis, to prevent mother-to-child transmission and to prevent re-infection in liver transplant patients. To understand the mechanisms that lead to chronic HBV infection and to new therapeutic approaches we have undertaken a systematic analysis of the T and B cell response to HBV antigens in acutely infected and resolving individuals and in chronic carriers. Specific T cells are characterized using the T cell libraries representing different types of memory and activated T cells, which are screened with overlapping peptides and recombinant molecules presented by different types of antigen presenting cells. Human monoclonal antibodies to S and preS1 antigens with neutralizing activity and breadth against different HBV genotypes and mutants are tested for their capacity to facilitate antigen presentation to T cells. We speculate that antibodies, by facilitating antigen presentation and reducing viral load, may boost host T and B cell responses thus contributing to seroconversion.

This work is done in collaboration with Federica Sallusto, IRB; Pietro Andreone, University of Bologna (IT); Florian Bihl, EOC Bellinzona (CH); and Humabs BioMed SA, Bellinzona (CH).

Anti-cancer antibodies from the human naïve B cells

Matteo Mauri and Antonio Lanzavecchia

The induction of primary B cell responses in vitro from naïve B cells has been a long sought goal but to date there are no reliable methods available. A potential application of an in vitro priming method would be the isolation of antibodies specific for tumor cells. We expect that there is no B cell tolerance to most tumor antigens, since these are not present during B cell development. Consequently anti-tumor B cells should be present, albeit at low frequencies, in the naïve B cell repertoire. Our aim is to obtain proof of concept that antibodies to tumors can be isolated from the naïve repertoire and subsequently use this observation to try developing an in vitro primary immune response model. Tumor cell lines were labeled with a fluorescent dye and incubated with naïve B cells and the rare B cells that have taken up antigens from tumor cells through trogocytosis were sorted and immortalized with high efficiency under conditions that promote isotype switch to IgG. The antibodies produced were screened for their capacity to stain tumor cells using secondary antibodies against IgM or IgG or for their capacity to lyse tumor cells in the presence of complement. Several antibodies reactive with tumor cell lines and some with complement-dependent cytotoxic activity were isolated and are currently characterized to identify the nature of the target antigen. Once the existence of tumor-specific naïve B cells is established, we will attempt to use this system to achieve a more ambitious goal, namely to induce an antigen-specific B cell response in vitro that would recapitulate the key events, including isotype switch, somatic mutations and selection of high affinity clones that occurs in vivo in the germinal center reaction.

This work was done in collaboration with Vito Pistoia, Istituto Gaslini, Genova (IT) and with Elisabetta Cameroni and Davide Corti, Humabs BioMed, Bellinzona (CH).

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

Antonio Lanzavecchia and Federica Sallusto

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

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*** *Zielinski C. et al.***
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Funding

CARIPLO Foundation
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CARIPLO Foundation
Human monoclonal antibodies as vaccine adjuvants and TLR agonist combinations as vaccine adjuvants / 2010-2013

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ABIRISK: Anti-Biopharmaceutical Immunization: Prediction and analysis of clinical relevance to minimize the risk
IMI-2010- Call3- 115303 / 2012-2017

European Union
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FP7-HEALTH-F3-2008-201169 / 2008-2012

European Union
IDAMS: International Research Consortium on Dengue Risk Assessment, Management and Surveillance
FP7-HEALTH-F3-2011-281803 / 2011-2016

European Union - ERC
IMMUNExplore: New approaches to analyze and exploit the human B and T cell response against viruses
ERC-2009-AdG-20090506-250348 / 2010-2015

European Union
ADITEC: Advanced Immunization Technologies
FP7-HEALTH-2011-280873 / 2011-2016

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U19 AI057266-06 / 2009-2014

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Susceptibility and protective immunity to Noroviruses
2R01AI056351-06A1-#5-30027 / 2009-2014

Swiss National Science Foundation
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31003A-126027/1 / 2009-2012

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Publications

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Lectures and Seminars

Institute Pasteur Vaccinology Course
Immunological memory: the challenge of conferring long-term protection?
Paris (FR) / 07.03.2012

PReS Course: Translational and Clinical Issues in Pediatric Rheumatology
Analysis of human T and B cells repertoires
Genoa (IT) / 08.03.2012

Keystone Symposia on Mutations, Malignancy and Memory: Antibodies and Immunity
Antibody Repertoire in Memory B Cells
Boston (US) / 21.03.2012

Seconda Giornata della Ricerca Clinica della Svizzera Italiana
Ricerca di base e traslazionale: quali prospettive in Ticino?
Bellinzona (CH) / 23.03.2012

BioBusiness: Advanced short course on BioEntrepreneurship at USI
Human Monoclonal Antibodies: the Humabs Case
Lugano (CH) / 07.05.2012

EMBO/EMBL Symposium: New Perspectives on Immunity to Infection
Dissecting the human T and B cell response to pathogens
Heidelberg (DE) / 19.05.2012

Association for Cancer Immunotherapy (CIMT) Annual Meeting 2012
Dissecting the human immune response to pathogens
Mainz (DE) / 24.05.2012

Nature Medicine Meeting: The Herrenhausen Symposium on Viral Pathogenesis
Mining the human memory T and B cell repertoires for immunotherapy and vaccine design
Kloster Seeon (DE) / 06.06.2012

Gordon Research Conference: Immunochemistry & Immunobiology
The human immune response to pathogens
Les Diablerets (CH) / 14.06.2012

NIAID/FDA Universal Influenza Vaccines Meeting
Panel speaker during Session 1: Scientific Questions
Bethesda (US) / 19.06.2012

Herpes virus infection and immunity conference
Development of monoclonal antibodies and T cells for immunotherapy against herpes
Veyrier du Lac (FR) / 20.06.2012

Convegno dell'Agenzia nazionale per la biofisica
informazione - Comunicazione cellulare: realtà e prospettive
Comunicazione cellulare: prospettive e ricerca applicata
Rome (IT) / 28.06.2012

6ème Journée Scientifique Miltenyi Biotec: Modulating innate and adaptive immunity in cancer
High-throughput screening of B cell repertoires
Paris (FR) / 29.06.2012

Swiss Biotech Roundtable: Next-generation antibodies on the verge of therapeutic breakthroughs
The impact of monoclonal antibodies on human health: current status and future trends
Rüschlikon (CH) / 09.07.2012

Cell Symposia: Human Immunity
Dissecting the human antibody response to pathogens and self antigens
Lisbon (PT) / 21.08.2012

Life Science Symposium 2012: Global Health meets Infection Biology
Dissecting the human T and B cell response to pathogens
Lausanne (CH) / 31.08.2012

3rd European Congress of Immunology 2012
Dissecting the human T and B cell response to pathogens
Glasgow (UK) / 06.09.2012

Kishimoto Foundation Lecture at Osaka University
Dissecting the human immune response to pathogens and self antigens
Osaka (JP) / 10.09.2012

The 11th Awaji International Forum on Infection and Immunity
Dissecting the human B and T cell response to pathogens
Awaji Island (JP) / 13.09.2012

11th National Congress of the Italian Society for Virology
Dissecting the human T and B cell response to pathogens
Orvieto (IT) / 18.09.2012

19th International Symposium on Hepatitis C Virus and Related Viruses
Dissecting the human T and B cell response to pathogens
Venice (IT) / 05.10.2012

International Symposium on Regulators of the Humoral Immune Response
Dissecting the human B cell response to pathogens and self antigens
Erlangen (DE) / 07.10.2012

Vienna Biocenter Seminar at the Research Institute of Molecular Pathology
Dissecting the human T and B cell response to pathogens
Vienna (AT) / 25.10.2012

Symposium: The impact of molecular biology on biotechnology and society
Immunology: Achievements and Visions
Zurich (CH) / 02.11.2012

Les Cent Gardes: HIV Vaccine Conference – The B cell response to HIV and HIV Vaccines: From broadly neutralizing to non-neutralizing antibodies
Dissecting the human B cell response to pathogens
Veyrier du Lac (FR) / 05.11.2012

21st Annual Norman Heatley Lecture
Dissecting the human immune response to pathogens and self antigens
Oxford (UK) / 08.11.2012

Seminar at the Department for Molecular Biomedical Research (DMBR)
Broadly neutralizing antiviral antibodies
Ghent (BE) / 16.11.2012

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 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. More recently, the group has proposed the concept of ERAD tuning, which asserts that post-translational events (e.g. regulated turnover of ERAD factors, changes in their sub-compartmental distribution and their participation in functional complexes) controlled by the level of misfolded protein in the ER are involved in the maintenance of cellular proteostasis by determining the overall ERAD capacity. Maurizio 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 he is Adjunct Professor at the EPFL. September 2012 he has been nominated commissary for chemistry and biology teaching at the High Schools in Cantone Ticino and since January 2013 he is member of the Research Committee at the Università della Svizzera Italiana.

Research Focus

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

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Substrate-Specific Mechanisms of Protein Degradation from the ER

Giorgia Brambilla Pisoni, Elisa Fasana, Jessica Merulla, Tatiana Soldà and Maurizio Molinari

Misfolded polypeptides produced in the ER are dislocated across the ER membrane to be degraded by cytosolic 26S-proteasomes in processes collectively termed ER-associated degradation (ERAD). Dislocation across the ER membrane is regulated by multimeric complexes built around one of the several membrane-embedded E3 ubiquitin ligases expressed in the mammalian ER. Physico-chemical features of the misfolded polypeptide (e.g. presence/absence of N-linked oligosaccharides, disulfide bonds, peptidyl-prolyl bonds in the cis conformation, membrane-anchor) may determine the quality control machineries that deliver the misfolded polypeptide at specific dislocation complexes. The definition of the rules that govern protein biogenesis and quality control requires a systematic analysis of appositely designed model folding-competent and folding-defective proteins. We have therefore prepared more than 50 model substrates with select physico-chemical features (Figure 1), whose fate will be monitored in mammalian cultured cells. Initially, we will investigate, as one example, the role of charged residues in polypeptide membrane-anchors. Preliminary results showed that the presence of charged residues in the transmembrane domain represents an additional ER degradation signal accelerating the disposal of the model ERAD substrates. Immunoisolation of the model substrate and analysis of the interacting ER-resident factors revealed specific association with known (i.e., SEL1L, HERP, HRD1) and new (i.e. TMX1) ERAD factors (unpublished data) (Figure 2). Overexpression of dominant negative mutants and/or knockdown of the interacting ERAD factors will be performed to evaluate their role in degradation of the selected model substrates.

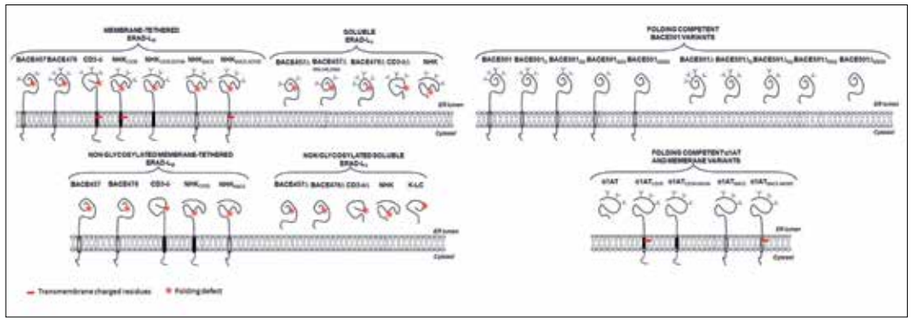

Figure 1.
Model substrates.
A collection of glycosylated and not glycosylated variants of membrane anchored (ERAD-L_M) and soluble (ERAD-L_S) folding-defective and folding-competent polypeptides used in our lab to investigate mechanisms of protein folding, quality control and degradation.

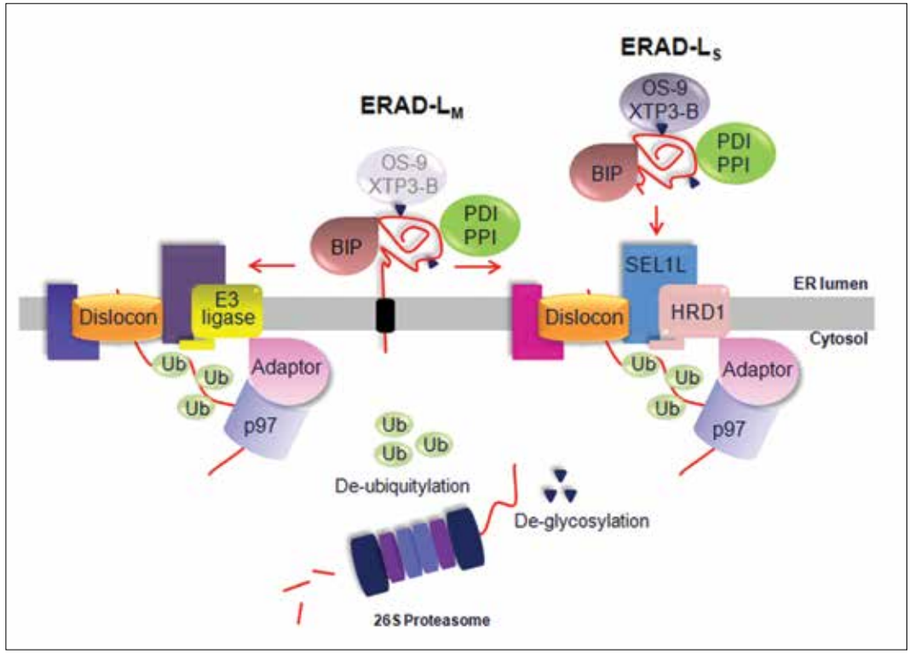

Figure 2.
Substrate-specific ERAD pathways.
The same misfolded module shows different requirements for efficient clearance from the ER when anchored to (ERAD-L_M substrate) and when detached from the membrane (ERAD-L_S substrate), the latter showing stronger dependency on ERAD lectins and HRD1 dislocon. Consistently, OS-9 and XTP3-B are depicted in lighter colors for ERAD-LM substrates. Red arrows show that ERAD-LM substrates can diffuse in the lipid bilayer to enter HRD1 or alternative dislocons.

Comparative Interactomics to Identify Novel ER-Resident Quality Control Players

Jessica Merulla, Tatiana Soldà 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 generated a series of stable human cell lines expressing epitope-tagged folding-competent and folding-defective proteins. The model proteins are used as baits to capture interacting partners in the same immuno-complexes. The proteins co-immunoisolated with the individual baits are subjected to tryptic digestion and fragments are separated by nano-HPLC followed by tandem mass spectrometry. Fragmentation spectra of the samples are matched to a human protein database sequence with the Mascot software. These analytic steps are performed in collaboration with Manfredo Quadroni, coordinator of the Center for Integrative Genomics, University of Lausanne. Involvement in protein quality control of

the interacting partners of the model proteins will be validated in 2 steps: i) confirmation of interaction by co-immunoisolation followed by Western blot; ii) evaluation of the role of the interactors by monitoring consequences on the substrate fate upon silencing of their expression or upon co-expression with the model substrate of their dominant negative mutants.

Disposal of Non-Glycosylated Polypeptides from the Mammalian ER

Elisa Fasana, Jessica Merulla, Tatiana Soldà and Maurizio Molinari

To maintain ER homeostasis and to ensure the highest efficiency of functional polypeptide production, the quality control machinery operating in the mammalian ER must distinguish non-native intermediates of protein folding programs from terminally misfolded polypeptides. Folding intermediates must be retained in the ER to attain the native structure under the assistance of dedicated molecular chaperones and folding enzymes. Terminally misfolded polypeptides must be, on the other hand, rapidly cleared from the ER lumen to avoid interferences with ongoing folding programs. For glycosylated polypeptides, which represent the majority of the cargo entering the secretory pathway, the processing of the N-linked oligosaccharides determines retention in the folding environment (cycles of removal/re-addition of terminal glucose residues) or extraction from the folding environment for disposal. Virtually nothing is known about quality control of non-glycosylated polypeptides. The aim of this project is the identification of ER-resident factors involved in quality control and disposal of both soluble and membrane-bound non-glycosylated variants of model glycopolypeptides generated in our lab. Main objectives are the characterization of their different degradation pathways and to understand which ER-resident quality control factors are eventually involved in their disposal.

Role of TMX1 in ERAD

Giorgia Brambilla Pisoni, Elisa Fasana and Maurizio Molinari

TMX1 is as a type I transmembrane ER-resident protein belonging to the protein disulfide isomerase (PDI) family. PDI family members assist the folding of newly synthesized proteins by catalyzing the oxidation, reduction and isomerisation of disulfide bonds. Members of the PDI family, namely reductases, play crucial roles in ERAD by breaking the covalent bonds between cysteines in the same (intra-molecular) or in different polypeptide chains (inter-molecular disulfide bonds). PDIs contain the characteristic CXXC active-site motif, with the two middle residues involved in determining the redox potential of the enzyme. TMX1 shows a unique active site (CPAC, residues 56-59) with the inner proline suggesting a role of the enzyme as a disulfide reductase. Recent evidences have shown that TMX1 reduces insulin disulfides and catalyzes refolding of scrambled RNase in vitro. Most importantly, TMX1 has recently been reported to regulate cell invasion by bacterial toxins that hijack the ERAD machinery to invade the host cell cytosol. This led us to postulate a possible intervention of TMX1 as ERAD reductase. This hypothesis will be verified by assessing a possible role of TMX1 in the clearance of our collection of model folding-defective substrates from the ER (Figure 1). In collaboration with L. Ruddock (Oulu University), substrate-binding and catalytic TMX1 mutants have been designed to aid us in characterizing its function.

ERAD Tuning: Regulation of the ERAD Activity in Mammalian Cells

Riccardo Bernasconi, Carmela Galli, Julia Noack and Maurizio Molinari

Bernasconi R. et al.*
Mol Cell. 2012, 46(6):809-19.

Adaptation of the ER folding and degradation activities to long-lasting changes in cargo load is regulated at the transcriptional level by activation of the unfolded protein response (UPR). UPR activation has a latency period of several hours. Hence, it is unsuited to rapidly respond to fluctuations in misfolded proteins load in the ER. Post-translational mechanisms have much shorter latency, since they do not depend on gene transcription and translation.
In particular, we showed that at steady state the complex comprising the type-I ER protein SEL1L and the cytosolic protein LC3-I acts as an ERAD tuning receptor regulating the COPII-independent vesicle-mediated removal of the luminal ERAD regulators EDEM1 and OS-9 from the ER (Figure 3). Luminal expression of folding-defective polypeptides enhances the content of EDEM1 and OS-9 by inhibiting their SEL1L:LC3-I-mediated clearance from the ER thereby selectively rising ERAD activity in the absence of UPR induction. The aim of this project is to identify the chaperones/enzymes whose intraluminal level is regulated by ERAD tuning, and to characterize the mechanisms regulating their segregation from long-lived chaperones that are retained in the bulk ER. Since the vesicular export of select ERAD factors from the ER is hijacked by pathogens (see next project), the characterization of the mechanisms regulating ERAD tuning and the identification of the cellular proteins involved in this process might lead to the identification of potential targets for anti-viral therapies.

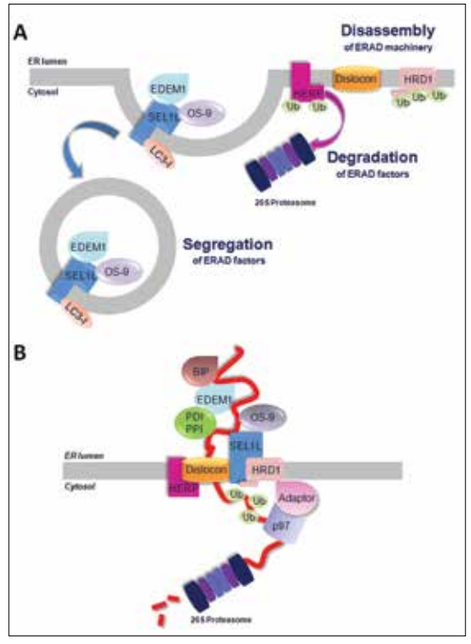

Figure 3
ERAD regulation by misfolded proteins.
A) *In healthy cells, unused dislocation machineries are disassembled. E3 ubiquitin ligases may for example poly-ubiquitylate themselves or components of the supramolecular complexes thereby resulting in their degradation. Orphan components may also be segregated from the ER lumen as shown by the vesicle-mediated release of SEL1L, EDEM1 and OS-9.*
B) *Misfolded proteins engage ERAD factors thereby preserving ERAD complexes, delaying ERAD factors turnover and segregation from the ER.*

ERAD Tuning: Hijacking by Viral Pathogens

Riccardo Bernasconi and Maurizio Molinari

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

* ***Bernasconi R. et al.***
Mol Cell. 2012, 46(6):809-19.

* ***Bernasconi R. et al.***
Autophagy. 2012, 8(10):1534-6.

Mechanisms Regulating the Recovery from Acute ER Stress in Mammalian Cells

Julia Noack, Carmela Galli and Maurizio Molinari

Expression of misfolded polypeptides, perturbations in the ER environment or dramatic changes in the ER cargo load may result in induction of the UPR. A series of stress sensors transduces the stress signal across the ER membrane thereby causing a global inhibition of transcription and translation, the selective up-regulation of folding and ERAD factors and the swelling of the ER upon enhanced lipid-biogenesis. The aim of this project is to establish how cells return to the "steady state situation" after having experienced a phase of acute stress that resulted in the transient enhancement of folding and ERAD factors concentration in the ER lumen. In particular, we are assessing how cells re-establish the steady state intraluminal concentrations of molecular chaperones and enzymes during the recovery phase. Preliminary data show that in the recovery phase after drug-induced ER stress, the stress-induced proteins return to their initial levels with very distinct kinetics indicating different, specific recovery mechanisms. Our data reveal that unstable proteins like HERP are removed by the proteasome, rapidly returning at their initial level (Figure 4). On the contrary, the recovery of stable proteins such as BiP involves dilution by cell division and/or ATG7-dependent autophagic processes (Figure 4). We will determine if an autophagy like mechanism (i.e. ER-phagy) takes place under the condition of stress recovery and set out to characterize this poorly defined mechanism in mammalian cells by i) immunofluorescent and ultrastructural analysis (in collaboration with F. Reggiori, Utrecht University), ii) purification of autophagosomes from isopycnic density gradients and mass-spectrometric analysis of their protein content and iii) identification of essential factors for ER-phagy by siRNA-mediated knockdown of candidate autophagy players. Importantly, we will establish a mammalian cell system to study recovery from ER stress directly induced by tunable expression of misfolded model substrates.

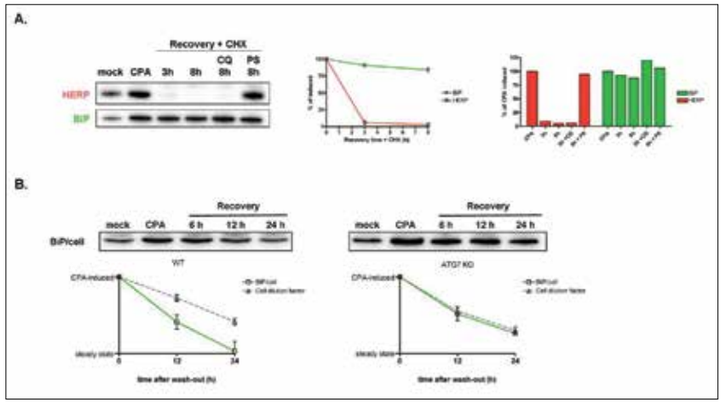

Figure 4
Recovery from ER stress.
A) *Acute ER stress was induced in mouse embryonic fibroblasts (MEFs) with cyclopiazonic acid (CPA). CPA was washed out to allow cells to recover from the ER stress in the presence of the protein biosynthesis inhibitor cycloheximide (CHX) and the lysosomal inhibitor chloroquine (CQ) or the proteasomal inhibitor PS-341 (PS). Relative protein levels of the ER-stress induced proteins HERP and BiP were determined by Western blot.*
B) *ER stress was induced by CPA in MEF wild type cells (wt) and MEF ATG7 KO cells. Cells were allowed to recover from the ER stress after CPA wash-out and cell numbers as well as BiP protein levels were determined. The protein levels of BiP (green lines) are compared with the dilution factor given by cell division (grey dashed lines).*

Funding

Swiss National Science Foundation
Protein folding, quality control and degradation in the ER
3100A0-121926/2002-2014

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

Novartis Stiftung für Medizinisch Biologische Forschung
ERAD tuning
2012

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

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

Association Française contre les Myopathies
Processing of disease-causing sarcoglycan mutant
MNM2Physiopath/2010-2012

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Publications

Specificity and Regulation of the Endoplasmic Reticulum-Associated Degradation Machinery.
Merulla, J., E. Fasana, T. Solda and M. Molinari.
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Role of the SEL1L:LC3-I Complex as an ERAD Tuning Receptor in the Mammalian ER.
Bernasconi, R., C. Galli, J. Noack, S. Bianchi, C. A. de Haan, F. Reggiori and M. Molinari.
Mol Cell. 2012; 46:809-819.

Lectures and Seminars

Molecular Chaperones and Stress Responses
ERAD tuning: mechanism and coronavirus hijacking (R. Bernasconi)
Cold Spring Harbor (US) / 1-5.05.2012

Basic Virology Course, Institut Pasteur
From protein folding to … how coronaviruses hijack the host cell ERAD tuning machinery for replication (R. Bernasconi)
Paris (FR) / 02.09.2012

Horizons in Molecular Biology 2012
Mechanisms regulating the recovery from acute ER stress in mammalian cells (J. Noack)
Goettingen (DE) / 8-11.10.2012

EMBO Conference 2012, Towards a comprehensive understanding of endoplasmic reticulum functions
ERAD tuning: setting ERAD activity by regulating ERAD factors segregation, integration in functional complexes and turnover
Girona (ES) / 15-19.10.2012

University of Zurich, Institute of Virology
Hijacking of the ERAD tuning machinery by Coronaviruses
Zurich (CH) / 23.11.2012

Organization of international congresses

Tenth Calreticulin Workshop
Banff (CA) / 10-13.04.2013

Silvia Monticelli

Molecular Immunology

Silvia Monticelli

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

Research Focus

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

Team

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Members: Lorenzo Dehò, PhD student - Nicole Rusca, PhD student - Sara Montagner, PhD student - Cristina Leoni, Technician

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

Nicole Rusca, Lorenzo Dehò, Sara Montagner and Silvia Monticelli

Mice that lack the transcription factor NF-κB1 are unable to mount eosinophilic airway inflammation due to impaired differentiation of lymphocytes to the Th2 subset, and consequently reduced expression of the cytokines IL-4, IL-5 and IL-13. Mast cells are important effector cells in asthma and allergic diseases and can produce high amounts of Th2-type cytokines. We therefore investigated whether a defect in mast cell differentiation and/or function could contribute to the lack of airway inflammation observed in these mice.
We found that bone marrow-derived mast cells (BMMCs) from NF-κB1-deficient mice showed a strong resistance to apoptosis in response to withdrawal of essential cytokines. By further investigating the role of NF-κB1 in influencing mast cell survival, we found that BMMCs lacking NF-κB1 were severely impaired in their ability to induce expression of miR-146a, and that this miRNA plays an important role in modulating mast cell survival. Indeed, forced miR-146a expression in primary BMMCs led to an increase in cell death, pointing towards a molecular network involving both NF-κB1 and miR-146a in regulating cell survival in mast cells. Moreover, miR-146a upregulation contributed to negatively regulate NF-κB activation through the down-modulation of the signalling molecules IRAK1 and TRAF6.
While the asthma-resistant phenotype of NF-κB1-deficient animals remains primarily Th2-dependent, we identified a novel molecular network that regulates mast cell survival and homeostasis in the tissues, which could be important in diseases related to abnormal accumulation of mast cells such as mastocytosis.

*** *Rusca N et al.***
Mol Cell Biol. 2012, 32:4432-4444

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

Sara Montagner, Cristina Leoni, Lorenzo Dehò and Silvia Monticelli

The prognosis for systemic mastocytosis is highly dependent on the type: although indolent mastocytosis is generally not fatal, other forms (e.g. mast cell leukemia and aggressive mastocytosis) usually have a rapidly devastating outcome. While mast cells are normally present in tissues exposed to the environment like mucosal tissues in the gut, in patients with systemic mastocytosis other organs, and most notably the bone marrow, become abnormally invaded by pathogenic mast cells. Despite that, the pathogenetic factors that contribute to mastocytosis variants are still largely obscure. For example, mastocytosis may occur also in absence of mutations in the oncogene *KIT*, indicating that the molecular reasons behind this disease is variable, it does not depend on a single mutation or oncoprotein, and it could have both genetic and epigenetic basis. We are investigating new genetic mutations in *KIT*, as well as in newly described oncogenes, that may lead to epigenetic modifications of gene expression (specifically, alterations of miRNA regulation and abnormal patters of DNA methylation) and ultimately to tumorigenic transformation. In particular, we are interested in understanding the role of DNA methylation and hydroxymethylation in mast cell functions and mast cell-related diseases (Figure 1).

*** *Montagner S et al.***
Immunol. Rev. 2013, 253:12-24.

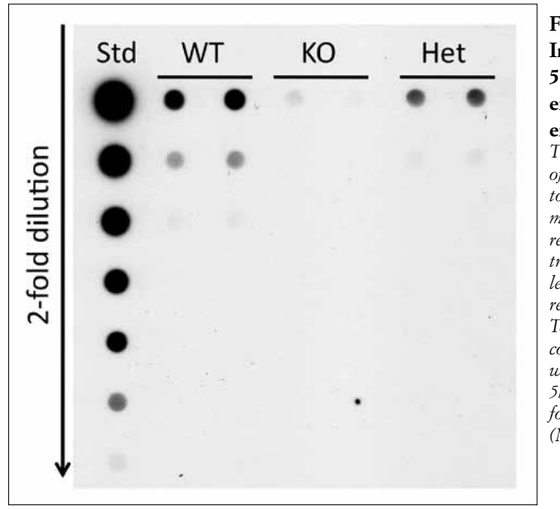

Figure 1.
In mast cells, levels of genomic DNA 5'-hydroxymethylcytosine are dependent on the expression of the dioxygenase enzyme Tet2
The enzyme Tet2 is responsible for the oxidation of 5-methylcytosine (5mC) in genomic DNA to 5-hydroxymethylcytosine (5hmC), and such modification contributed to gene transcriptional regulation and in some cases to tumorigenic transformation. In mouse mast cells, overall levels of 5hmC in genomic DNA are strongly reduced in cells lacking completely the gene for Tet2 (KO), or having only one allele (HET), compared to control (WT) cells. Levels of 5hmC were measured upon bisulfite conversion of 5hmC to cytosine 5-methylenesulfonate (CMS), followed by detection with an anti-CMS antibody (Montagner S, unpublished). Std: standard.

Role of CD25 in normal mast cell functions and in mast cell-related proliferative disorders

Lorenzo Dehò, Cristina Leoni, Sara Montagner and Silvia Monticelli

In patients with systemic mastocytosis, the surface marker CD25 (IL-2Rα), which is one of the components for the interleukin-2 receptor (IL-2R), is specifically expressed on pathologic mast cells in the bone marrow. By immunohistochemistry, CD25 is both highly sensitive and specific for systemic mastocytosis, showing diffuse staining of virtually all neoplastic mast cells in nearly 100% of the cases, but no information is available about the role of IL-2 or the IL-2R in mast cell differentiation, biology, homeostasis or disease. IL-2 controls normal proliferation and differentiation of immune system cells; it also inhibits growth of certain human tumor cells while proliferation of others remains intact or is even stimulated. Changes in the expression of IL-2 and its receptor generally correlate with the rate of pathology development, although the complete biological role of CD25 expression in tumors is unclear. Recombinant IL-2 has found therapeutic application for treatment of patients with renal carcinoma, acute leukemia and melanoma. Clinical treatments use various components of the IL-2/ IL-2R complex or their antagonists, and it remains therefore essential to understand what is the role of IL-2 and its receptor in mast cell function, differentiation and disease. Our studies have identified specific roles for CD25 as a marker of a specific subset of normal mast cells, with characteristic patterns of proliferation and cytokine production, and shed more light on the potential use of CD25 as a target for therapy.

Transcription factors and microRNAs in the regulation of human TH17 lymphocytes

Federico Mele, Cristina Leoni, Federica Sallusto and Silvia Monticelli

T lymphocytes are components of the immune system, essential to orchestrate responses to invading pathogens, and the immune response to different microorganisms is tailored by the differentiation of CD4 T helper (Th) cells to different subtypes. Different Th lineages, such as Th1, Th2 and Th17, are characterized by the expression of signature cytokines, such as IL-4 for Th2 and IL-17 for Th17 cells. Th17 cells in particular participate in host defence against fungi and extracellular bacteria. We are interested in investigating the mechanisms underlying IL-17 production and Th17 activation in response to antigens, and following up on previous work (Zielinski et al. Nature 2012), we identified a novel molecular network comprising transcription factors and microRNAs involved in the modulation of T cell receptor signal strength specifically in Th17 cells.

* ***Zielinski CE et al.***
Nature. 2012, 484:514-518.

Funding

Swiss National Science Foundation
Analysis of novel molecular networks underlying mast cell development, function and disease: specific roles of CD25, NF-kB p50 and microRNAs
31003A_138343
2012-2015

Sassella-Stiftung Award
2012

Ceresio Foundation
2011-2013

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Hematology Clinic, Fondazione IRCCS Policlinico San Matteo, Pavia (IT)

Visiting Scientists

Marco De Simone
Istituto Nazionale di Genetica Molecolare, Milano (IT)

Publications

Pathogen-induced human TH17 cells produce IFN-gamma or IL-10 and are regulated by IL-1beta.
Zielinski, C. E., F. Mele, D. Aschenbrenner, D. Jarrossay, F. Ronchi, M. Gattorno, S. Monticelli, A. Lanzavecchia and F. Sallusto.
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Negative regulators take center stage.
Monticelli, S. and F. Sallusto.
Nat Immunol. 2012; 13:719-720.

Lectures and Seminars

National Institute for Molecular Genetics (INGM)
Milan (IT) / 01.03.2012

Tessin Society for Allergy and Immunology (STAI)
Bellinzona (CH) / 15.03.2012

World Immune Regulation Meeting VI, "Innate and Adaptive Immune Response and Role of Tissues in Immune Regulation" March 18-21, 2012
Davos (CH) / 19.03.2012

University of California (UCSF)
San Francisco, CA (US) / 25.05.2012

EADV meeting: Immune response in chronic inflammatory and autoimmune diseases
Bellinzona (CH) / 08.11.2012

International Mast Cell and Basophil meeting
Berlin (DE) / 26.11.2012

University of Arizona
Tucson, AZ (US) / 12.12.2012

La Jolla Institute for Allergy and Immunology
San Diego, CA (US) / 14.12.2012

Federica Sallusto
Cellular Immunology

Federica Sallusto

Federica Sallusto received the degree of Doctor in Biology from the University of Rome and performed post-doctoral training at the Istituto Superiore di Sanità in Rome working on T cell response to allergens and at the Basel Institute for Immunology in the laboratory of Antonio Lanzavecchia on human monocyte-derived dendritic cells. In 1997 she became member of the Basel Institute and since 2000 she is a goup leader at the IRB. Her studies in the human system revealed a differential expression of chemokine receptors in Th1 and Th2 cells and led to the characterization of "central memory" and "effector memory" T cells as memory subsets with distinct migratory capacity and effector function. Among her recent contributions are the discovery of Th22 cells, the identification of markers to identify Th17 cells and the characterization of two distinct types of pathogen-specific human Th17 cells that produce IFN-γ or IL-10. Studies in the mouse model challenged current dogmas as to the mechanisms that control lymphocyte migration in lymph nodes and in the central nervous system; more recently her lab showed that persistent antigen and germinal centre B cells sustain Tfh cell responses and phenotype. For her scientific achievements, Federica Sallusto received the Pharmacia Allergy Research Foundation Award in 1999, the Behring Lecture Prize in 2009, and the Science Award from the Foundation for Studies of Neurodegenerative Diseases in 2010. She was elected member of the German Academy of Science Leopoldina in 2009 and member of EMBO in 2011. She is President of the Swiss Society for Allergology and Immunology for the period 2013-2015.

Research Focus

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

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

Christina E. Zielinski, Federico Mele, Dominik Aschenbrenner, David Jarrossay, Francesca Ronchi, and Federica Sallusto*
**Former postdoc*

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

*** *Zielinski C.E., et al.***
Nature 2012, 484:514-518.

*** *Sallusto, F., et al.***
Eur J Immunol. 2012; 42:2215-2220.

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

Deciphering the class and specificity of the T cell response to *C. albicans*

Simone Becattini, Gabor Gyülvészi, Laurent Perez and Federica Sallusto

From the immune system point of view, microbes (pathogens or commensals) are complex antigens that occupy distinct niches and consequently trigger different types of immune responses. The complexity of the microbial proteome, in particular that of bacteria and fungi, represents a considerable challenge to our capacity to analyse the human T cell response. We are using complementary approaches in order to study the human T cell response to *Candida albicans* and to identify immunodominant and protective antigens. On the one hand, we are performing a wide screening of HLA-binding peptides, identified through bioinformatic analysis, from 80 fungal proteins belonging to different classes for their capacity to be recognized by different memory T cell subsets. On the other hand, we isolate and identify proteins contained in cell wall extracts that are recognized by human IL-17-producing memory T cells (Th17) and that induce strong immune responses when used as vaccines in mice. These studies are expected to improve our understanding of the immune response to complex pathogens, to define the correlation between class of antigens and type of T cell response elicited, and, finally, to provide useful information for the design of subunit vaccines against *C. albicans*.

This work is done in collaboration with Alessandro Sette, La Jolla Institute for Allergy and Immunology, La Jolla, CA (US) and Nico Callewaert, Ghent University (BE).

Unravelling the transcriptional circuit regulating IL-10 production in human Th17 cells

Samuele Notarbartolo, Dominik Aschenbrenner and Federica Sallusto

IL-17 producing CD4+ cells (Th17) are a subset of effector T helper cells known to play an important role in host defense against fungi and extracellular bacteria but also involved in tissue inflammation and autoimmune diseases, such as multiple sclerosis, inflammatory bowel disease, and rheumatoid arthritis. The function of Th17 cells depends critically on the range of cytokines produced and on the balance between pro- and anti-inflammatory cytokines. Autoreactive Th17 cells producing IFN-γ and GM-CSF are pathogenic in a mouse model of EAE, while Th17 cells producing IL-10 are not. We have recently shown that *C. albicans*-specific human Th17 cells produce IL-17 and IFN-γ, while *S. aureus*-specific human Th17 cells produce IL-17 and, after restimulation, IL-10. While the ontology of the two different Th17 subsets has been clarified, it still remains elusive what is the transcriptional circuit that regulates the expression of the immunoregulatory molecule IL-10. Using a combination of transcriptional profiling and epigenetic approach, we identified the transcription factor c-MAF as a candidate for the regulation of IL-10 production in human Th17 cells, thus potentially representing a discriminant factor between pathogenic and non pathogenic Th17 cells.

Regulation of cytokine production in human T helper cells

Dominik Aschenbrenner, Federico Mele, Samuele Notarbartolo, and Federica Sallusto

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

Development of a multiflow panel for the analysis of chemokine receptor expression on human T helper cells

Tess M. Brodie, Elena Brenna and Federica Sallusto

Chemokine receptors are used to identify different subsets of human and mouse memory T cells. A flow cytometry panel that identifies many combinations of chemokine receptors expressed on T cells is required in order to improve our understanding of the heterogeneity of T cell subsets and the role these subsets play in different types of protective or pathological immune responses. The strategy used to develop a chemokine

receptor panel was to first choosing the primary antigens that are best characterized and that identify broad subsets (CD3, CD4, CD14, CD16, CD19). The secondary antigens chosen were also high density antigens, but expressed more as a continuum (CD45RA, CCR7, and CD95). The tertiary antigens were the least expressed (CXCR3, CXCR5, CCR4, CCR3, CCR5, CCR6, CCR10, CRTh2) and therefore most difficult to detect. Dead cells were excluded from the analysis. With this organization, fluorochromes were assigned based on the brightness and availability for the tertiary antigens, leaving the rest of the detectors available for the well characterized antigens. To ascertain the best staining conditions, different types of samples were used and all anti-chemokine receptor antibodies were titrated at a range of temperatures (4°C, 18°C and 37°C), and stained for 10 to 20 minutes prior to acquisition. It was noted that the quality of chemokine receptor staining is affected by a variety of factors, most notably the time post blood draw, cryopreservation, whether samples were magnetically sorted prior to staining and temperature during staining. Optimal staining requires PBMCs to be processed and acquired within 5 hours of blood draw and may be performed in a 96-well plate format for high throughput experiments.

* ***Brodie, T., et al.***
Cytometry. 2013, 83:530-532.

Elena Brenna received the Master degree from the University of Milan (IT) with the thesis "Heterogeneity of the human T cell response to environmental allergens".

Proteome wide analysis of HIV specific naïve and memory CD4+ T cells in unexposed blood donors

Tess Brodie, Astrea Rossetti and Federica Sallusto*
**Former Technician*

It is likely that primary immune responses are influenced strongly by the pre-existing repertoire of B and T cells. However, characterization and quantification of these repertoires is difficult due to the extremely low number of circulating naive precursor cells. Most previous studies of naïve CD4+ T cell repertoires in humans and mice have relied on magnetic beads to enrich MHC tetramer positive cells. However, this approach is necessarily limited to particular known epitopes and HLA types, so while giving precise information on these responses it cannot yet measure the total repertoire and misses previously unknown epitopes. We developed an alternative approach - the T cell library technique - that requires no prior knowledge of donor HLA type or epitope specificity (Geiger R., et al., J Exp Med 2009). The method pre-sorts circulating T cells into naive and memory subsets which are seeded at limiting dilution (1,000-2,000 cells/well) prior to polyclonal expansion in the presence of PHA, allogeneic feeder cells and IL-2. Individual cultures are then screened for proliferative responses to a protein or series of peptides representing the pathogen of interest. In this study we adopted the T cell library technique to provide a proteome wide analysis of the frequencies and specificities of pre-exposure HIV-1 specific naïve and memory CD4+ T cells in a HLA diverse population of 10 HIV-1 unexposed, seronegative donors. HIV-1 specific T cells were readily detected in all subsets. They were present in the naïve subset, at a mean of 55 per million, and in the central and effector memory subsets at means of 38.9 and 34.1 cells per million, respectively. Strikingly, 88% of the epitopes recognized by the naïve T cells and 58% of the epitope recognized by memory T cells had previously been reported in natural HIV-1 infection. Moreover, 10% of mapped epitopes were detected in two or more donors irrespective of HLA type, consistent with a sizable proportion of promiscuous peptides in the pre-exposure CD4+ T cell repertoire. Together these results underline the power of an unbiased, proteome-wide analysis of pathogen recognition by human naïve T cells to identify immunodominant antigens and provide a baseline for optimizing HIV-1 specific helper cell responses by vaccination.

This work was done as a joint project of the IRB and the MRC Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital (UK) (Suzanne Campion, Nilu Goonetilleke and Andrew McMichael).

Distribution of *Mycobacterium tuberculosis*-specific CD4+ T cells within distinct human memory subsets

Federico Mele and Federica Sallusto

While several studies have reported the identification of *Mycobacterium tuberculosis* (MTB) antigens, from abundant or easily purified proteins, a truly genome-wide study to identify antigens is lacking. Another unresolved issue relating to MTB immunity is whether different classes of antigens elicit responses that have the same or diverse functional characteristics. MTB antigens described so far are predominantly secreted MTB proteins, some of which are not essential for bacterial survival. As a result, it was hypothesized that secreted proteins might act as decoy antigens, diverting the immune response from recognizing more relevant MTB proteins. In this study we used HLA class II peptide binding predictions, HLA class II multimers, and the screening of T cell libraries, to perform an unbiased, genome-wide analysis of the CD4 T cell response to MTB in latently infected individuals. We showed that human CD4 T cells specific for MTB are highly focused on three broadly immunodominant antigenic islands, all related to bacterial secretion systems, thus refuting the notion that secreted antigens act as a decoy, since both secreted proteins and proteins comprising the secretion system itself are targeted by a fully functional T cell response. In addition, several novel T cell antigens were identified which can be of potential diagnostic use, or as vaccine antigens. Using the T cell library technique we showed also that MTB-responding T cells were highly enriched in libraries derived from the CCR6+CXCR3+ T cell subset (enriched in Th1 cells), and present at lower frequency in libraries from the CCR6+CXCR3– (enriched in Th17 cells) and the CCR6– subset (containing both Th1 and Th2 cells). This pattern of distribution was remarkable consistent: in all 4 donors analyzed more than 80% of the MTB-reactive memory CD4 T cell response resided in the CXCR3+CCR6+ subset. We are currently determining the TCR repertoire diversity of MTB-specific T cells in different memory subsets in by deep sequencing in order to define the origin and lineage relationship of these phenotypically and functionally distinct T cell populations (Figure 1).

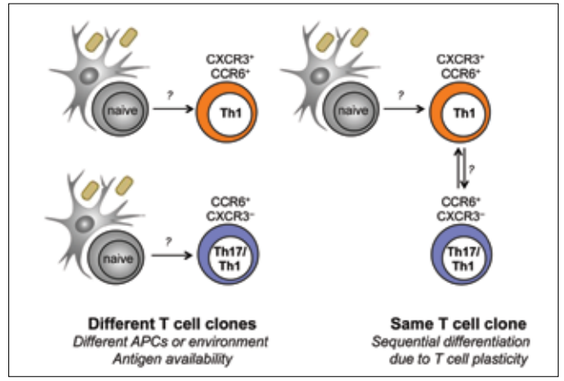

Figure 1.
Models for the generation of heterogeneous MTB-responding T cells

This work is shared between IRB and La Jolla Institute for Allergy and Immunology, La Jolla, California (US), and was done together with Cecilia S. Lindestam Arlehamn, Anna Gerasimova, Ryan Henderson,Justine Swann, Jason A. Greenbaum, Yohan Kim, John Sidney, Denise M. McKinney, Howard Grey, Bjoern Peters and Alessandro Sette.

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

Tess M. Brodie, Elena Brenna, and Federica Sallusto

Allergic sensitization occurs early in childhood upon allergen encounter in persons who have an inherited atopic predisposition. Environmental factors, route, period, and dose of allergen encountered influence the development of the allergic immune response. In the case of allergic individuals, T cell responses show a preferential Th2 phenotype that leads to the production of IgE antibodies, while non-allergic individuals respond to allergens with IgG production and a balanced Th1/Th2 phenotype. IgE is the least abundant class of immunoglobulins but can elicit immediate and strong inflammation through activation of mast cells and basophils via the high affinity receptor FcεRI. We are revisiting the response studying the dynamics of antigen responding CD4 T cells upon natural exposure to allergens in allergic and non-allergic donors, both in and out of allergy season. We are using novel high through put cellular screening approaches to dissect in great detail the phenotype and function of T cells responding to seasonal allergens, like ragweed and timothy grass, or to perennial allergens, such as house dust mite. We are comparing allergic to non-allergic donors to define frequency, class, and distribution in different subsets of the allergen-specific T cells. Since both phenotype and function are related to the location of the antigen challenge, the type and strength of costimulation and cytokines seen during T cell priming, this project will give us insights into the process of sensitization and clinical manifestations which are associated with different types of allergens.

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

Daniela Impellizzieri, Daniela Latorre and Federica Sallusto

Our aim is to develop sensitive methods to study self-reactive T cells in healthy donors and in patients with autoimmune diseases. Since self-reactive T cells may have low avidity and may be present at low frequencies we will combine cell sorting strategies, which highly enrich specific populations, with the T cell library method that allows detection of even low avidity cells. The feasibility of this approach is illustrated by the analysis of T cells from multiple sclerosis (MS) patients that shows that MOG-specific T cells are detectable in most patients and are virtually all present in the CCR6+ memory subset (Figure 2). These findings are consistent with our previous demonstration that CCR6 is required to drive migration of pathogenic T cells in the CNS of mice developing autoimmune encephalomyelitis. We are extending the analysis to other organ-specific autoimmune diseases, in particular pemphigus, for which autoantigens are well characterized, and to other conditions of aberrant responses to self antigens such as Pulmonary alveolar proteinosis, a rare disease characterized by autoantibodies against GM-CSF, and Factor VIII-treated hemofilia in which anti-Factor VIII antibodies develop in response to the therapy.

These studies are done in collaboration with Antonio Uccelli, University of Genova (IT) and Gianna Zambruno, IDI, Rome (IT).

*** *Sallusto, F., et al.***
Immunol. Rev. 2012, 248:216-227.

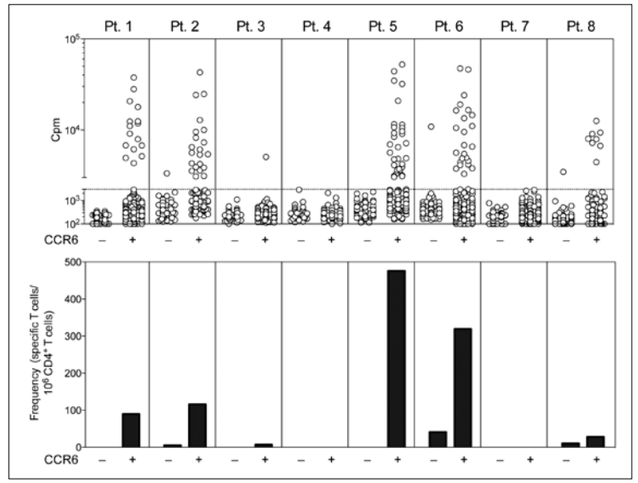

Figure 2.
MOG-reactive T cells in MS patients express CCR6. A. T cell libraries were generated from CCR6⁺ and CCR6⁻ memory T cells from eight MS patients (Pt.1-Pt.8). The libraries were screened with autologous monocytes pulsed with MOG and T-cell proliferation was evaluated using a ^{3}H-thymidine incorporation assay. The dotted line represents the cut off value. B. Frequencies of MOG-reactive T cells calculated on the basis of the fraction of negative cultures and the input of cells per culture. Values are expressed per 10^6 CD4⁺ T cells

Dissecting the naïve T cell repertoire – a way to define novel tumour antigens

Florian Wimmers and Federica Sallusto

Curing cancer has been the major goal of biomedical research in the last decade. Whereas some cancer types like chronic myeloid leukaemia are relatively easy to treat nowadays, others like melanoma or pancreatic adenocarcinoma are still related to a very poor prognosis. Recent studies on immunotherapy showed promising results by exploiting the patient's immune system to selectively eradicate tumour tissue. However, to make these vaccination strategies more efficient, it is of great importance to find antigens that are highly selective for tumour cells but also easily recognized by the T cells of the patient. So far there are only few studies published that systematically analyse the ability of T cells to recognise known tumour antigens. Most of these studies focus only on effector or memory T cells. Because of this, these studies fail to determine the whole potential of antigen recognition by the T cell repertoire, which includes in naïve T cells present only in healthy individuals. However, this repertoire is of great interest for further vaccination strategies, since it may largely determines the efficacy of the induced immune response. The aim of this project is, hence, to define the naïve T cell repertoire against a collection of common tumour antigens in healthy donors as well as in cancer patients before and after immunotherapy. The results of this study may have an impact on design of immunotherapies and help to improve the efficacy of this third line of cancer treatment.

T follicular helper cell response and phenotype are sustained by persistent antigen and germinal center B cells

Dirk Baumjohann, Silvia Preite, Andrea Reboldi*, Francesca Ronchi, and Federica Sallusto*
**Former PhD student*

T follicular helper (Tfh) cells provide help to B cells and are crucial for the establishment of the germinal center (GC) reaction, including the production of high-affinity antibodies and the generation of memory B cells and long-lived plasma cells. Here we report that the magnitude of the Tfh cell response was dictated by the amount of antigen and directly correlated with the magnitude of the GC B cell response. In addition, maintenance of the Tfh cell phenotype required sustained antigenic stimulation by GC B cells. In lymphopenic conditions, a strong and prolonged Tfh cell response led to bystander B cell activation, hypergammaglobulinemia, and production of poly- and self-reactive antibodies (Figure 3). These data demonstrate that antigen dose determines the size and duration of the Tfh cell response and GC reaction, highlight the transient nature of the Tfh cell phenotype, and suggest a link between overstimulation of Tfh cells and the development of dysregulated humoral immune responses.

*** *Baumjohann D., et al.***
Immunity 2013, 38: 596-605.

This work is done in collaboration with Antonio Lanzavecchia, IRB, and K. Mark Ansel, UCSF, CA (US).

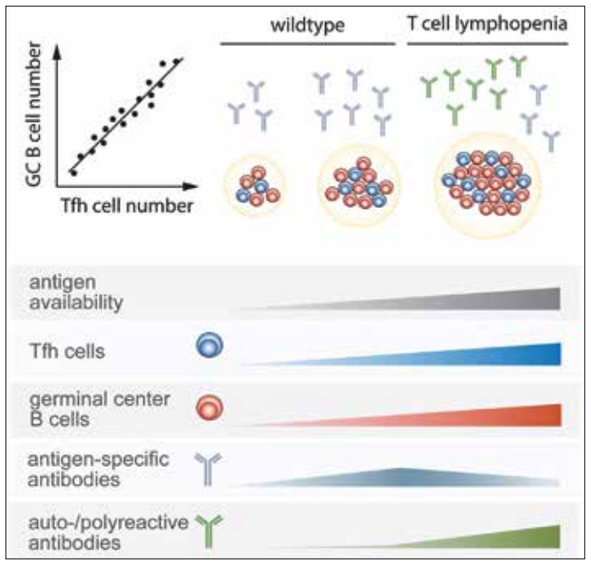

Figure 3.
Graphical representation of the results

T follicular helper cell response in lymphopenic mice

Silvia Preite, Luana Perlini and Federica Sallusto

In the previous study we showed that T cells in lymphopenic mice preferentially differentiate into Tfh cells which are however impaired in their ability to induce antibody affinity-maturation and to help generate long-lived plasma cells. Also, we showed that in lymphopenic mice a large number of monoclonal OVA-specific Tfh cells can induce activation of "bystander" B cells and production of isotype switched antibodies of unrelated specificities. To understand the basis of this phenomena, we are performing several experiments. First, we have cloned and sequenced Ig genes from NP-specific B cells 21 days following immunization of OT-II-transferred CD3ε$^{-/-}$ mice with NP-OVA. We found that two point mutations in the VH186.2 sequences, which are known to confer high affinity to anti-NP antibodies, were only present in wild type mice but not in CD3ε$^{-/-}$ mice, consistent with low serum antibody titers found in CD3ε$^{-/-}$ mice as measured by ELISA. Additional experiments are being performed to obtain sequences and identify somatic mutations that will be used to draw phylogenetic trees and define branch points. Based on a in house developed algorithm we hope to gain a better understanding on how and when antibody sequences differed in wild type and CD3ε$^{-/-}$ mice. Second, we are defining the requirements to induce the "bystander" B cell activation which is observed as a consequence of the strong and prolonged Tfh cell response in lymphopenic mice. Ongoing experiments are addressing the role of cognate interaction, for instance using B cells of unrelated specificities lacking MHC class II molecules. Finally, we are investigating the role of Tregs in controlling Tfh responses, in light of the recent findings of specialized Treg cells (Tfr) residing in the B cell follicle and regulating GC reactions. We found that Treg cells co-transferred with OT-II into CD3ε$^{-/-}$ mice immunized with OVA in alum become dysfunctional and lose the ability to suppress naïve CD4 T cells in vitro when isolated from CD3ε$^{-/-}$ hosts 22 days after transfer. Since some cytokines, for example IL-21 and IL-15, are known to drastically reduce the suppressive activity of Treg cells, we are planning to perform adoptive transfer experiments using Treg cells from mice deficient in different cytokine receptors. Experiments using Treg from DEREG-IL-21R$^{-/-}$ mice (kindly provided by Manfred Kopf, ETH Zurich) are ongoing.

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

Gabor Gyülvészi, Tomasz Wypych and Federica Sallusto

Genetic fate mapping provides means to evaluate cell lineage stability and information on mechanisms of cell maintenance *in vivo*. We are using this approach to evaluate Tfh lineage stability in primary and memory phases of the immune response by generating knock-in mice harbouring a cassette containing an internal ribosome entry site (IRES) followed by DNA sequence encoding a "triple" fusion protein, formed by enhanced green fluorescent protein (eGFP), Cre recombinase and mutated human estrogen receptor ligand-binding domain (ERT2), inserted into the 3' untranslated region (UTR) of the CXCR5 gene. The generation of the reporter mouse can be achieved through the traditional homologous recombination in ES cells or through the use of an innovative method, the TALEN nucleases. We decided to apply the second approach which enables us to omit the ES cell work and to directly inject the TALENs together with the targeting construct into oocytes. The advantage of this method is the increased recombination frequency and shorter time frame. We have already generated the targeting vector which contains the reporter sequence (IRES-

eGFP-CreErt2) and the two short homology arms which are required for proper integration. The reporter sequence was incorporated into the 3' UTR of the CXCR5 gene after the stop codon directly. We decided to keep the original 3' UTR polyadenylation signal in order not to interfere with the native integrity of the gene. The generated TALEN nuclease is currently being used for oocyte microinjection together with the plasmid. Once generated, the *Cxcr5eGfp-Cre*-ERT2 mice will be bred to mice that express a *Cre* recombination reporter allele of the ubiquitously expressed ROSA26 locus containing a loxP site–flanked stop-cassette followed by a DNA sequence encoding yellow fluorescent protein (YFP). In these mice, the GFPCreERT2 fusion protein will be sequestered in the cytosol, and therefore YFP will not be expressed. Treatment with tamoxifen will allow for nuclear translocation of the fusion protein, excision of the floxed stop-cassette, and constitutive and heritable expression of YFP in a population of cells that express CXCR5 at the time of tamoxifen administration.

This work is done in collaboration with Wolfgang Wurst and Ralf Kühn, Helmholtz Zentrum München (DE), and with Jens Stein and Britta Engelhardt, TKI, Bern (CH).

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

Camilla Basso, Luana Perlini and Federica Sallusto

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

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

Francesca Ronchi, Camilla Basso, Luana Perlini and Federica Sallusto

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

Innate and adaptive immune response to *C. albicans*

Gabor Gyülvészi, Simone Becattini, Luana Perlini and Federica Sallusto

C. albicans is part of the human commensal flora and poses no risk to healthy individuals. However, under certain circumstances it colonizes the vagina and develops into recurrent infection, affecting 70% of the female population. It is not known why this infection develops and how the immune system can control the pathogen in the vaginal tissue. Earlier studies showed that the IL-17 axis is a crucial part of the host defense mechanism against fungal infections in other tissues. T cells and γδ T cells were identified as being the major source of IL-17 in response to *C. albicans*. By using a mouse model of vaginal candidiasis, we found that protection in the vaginal tissue is not only dependent on T cells but it requires the presence of IL-22-producing innate lymphoid cells as well. Mice either deficient of IL-22 producing T cells (*Rag1*$^{-/-}$) or IL-22 producing ILCs (*Rorc*$^{-/-}$, *Il23a*$^{-/-}$) are unable to control candida infection. Interestingly, mice lacking ILCs are more susceptible and succumb earlier to candida infection than mice lacking only T cells. This observation reveals a so far undescribed interaction between the innate and adaptive arm of the immune system in the vaginal tissue, similar to the one observed in the gut mucosa. ILCs promptly produce IL-22 upon infection and most likely slow down the colonization of the vagina by candida. This first, antigen unspecific wave of immune response is followed by the activation of IL-22 producing T cells which ultimately leads to pathogen clearance. This work is done in collaboration with Burkhard Becher, University of Zurich (CH).

The role of B cells as antigen presenting cells in allergic inflammation

Tomasz Wypych and Federica Sallusto

Allergy is one of the leading health problems in industrialized countries, affecting around 50 million people in the United States only. The hallmark of this disorder is a strong Th2 response with upregulated levels of IL-4, IL-5 and IL-13, which leads to enhanced IgE production, cell recruitment to the site of allergen entry and exaggerated immune response leading to tissue damage. The pathological role of B cells in allergic disorders as the source of immunoglobulin E has been known for many years. In contrast, B cell capacity to regulate T helper cell responses via MHC class II dependent presentation of allergenic peptides in ongoing airway inflammation remains elusive. Using a mouse model of house dust mite induced allergic asthma, we seek to determine if B cells residing in lungs are able to stimulate effector T helper cells in the lungs to proliferate and secrete cytokines. This approach may help us determine if B cells may contribute to allergic inflammation via presentation of antigens to resting memory Th2 and/or effector Th2 cells during a secondary immune response.

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Publications

Therapeutic Efficacy of Antibodies Lacking FcgammaR against Lethal Dengue Virus Infection Is Due to Neutralizing Potency and Blocking of Enhancing Antibodies.
Williams, K. L., S. Sukupolvi-Petty, M. Beltramello, S. Johnson, F. Sallusto, A. Lanzavecchia, M. S. Diamond and E. Harris.
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miR-146a and NF-kappaB1 Regulate Mast Cell Survival and T Lymphocyte Differentiation.
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Mol Cell Biol. 2012; 32:4432-4444.

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Oseroff, C., J. Sidney, R. Vita, V. Tripple, D. M. McKinney, S. Southwood, T. M. Brodie, F. Sallusto, H. Grey, R. Alam, D. Broide, J. A. Greenbaum, R. Kolla, B. Peters and A. Sette.
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Monticelli, S. and F. Sallusto.
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Systems Analysis of MVA-C Induced Immune Response Reveals Its Significance as a Vaccine Candidate against HIV/AIDS of Clade C.
Gomez, C. E., B. Perdiguero, V. Jimenez, A. Filali-Mouhim, K. Ghneim, E. K. Haddad, E. D. Quakkerlaar, J. Delaloye, A. Harari, T. Roger, T. Dunhen, R. P. Sekaly, C. J. Melief, T. Calandra, F. Sallusto, A. Lanzavecchia, R. Wagner, G. Pantaleo and M. Esteban.
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Duhen, T., R. Duhen, A. Lanzavecchia, F. Sallusto and D. J. Campbell.
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Pemphigus autoantibodies generated through somatic mutations target the desmoglein-3 cis-interface.
Di Zenzo, G., G. Di Lullo, D. Corti, V. Calabresi, A. Sinistro, F. Vanzetta, B. Didona, G. Cianchini, M. Hertl, R. Eming, M. Amagai, B. Ohyama, T. Hashimoto, J. Sloostra, F. Sallusto, G. Zambruno and A. Lanzavecchia.
J Clin Invest. 2012; 122:3781-3790.

Lectures and Seminars

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

Keystone Symposium, Joint "The Biology of Cytokines/Th17 Cells in Health and Disease"
Keystone, CO (US) / 5-10.02.2012

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

Pediatric Rheumatology European Society Course "Translational and Clinical Issues in Pediatric Rheumatology"
Genoa (IT) / 7-10.03.2012

Biogen Idec Italia "Transforming Discovery Into Care". Keynote Lecture.
Milan (IT) / 15-16.03.2012

VI World Immune Regulation Meeting
Davos (CH) / 18-21.03.2012

Weizmann Institute of Science
Course Highlights in Immunology Lecture "T cell differentiation and plasticity"
Rehovot (IL) / 26.03.2012

Weizmann Institute of Science
Guest Seminar "Human T cell subsets in infection and inflammation"
Rehovot (IL) / 27.03.2012

Mérieux Foundation "Aging and Immunity II"
Siena (IT) / 22-24.04.2012

Catalan Society of Immunology, Day of Immunology Meeting
Barcelona (SP) / 25.04.2012

Gordon Research Conference on Chemotactic Cytokines
Il Ciocco, Lucca (IT) / 27.05-01.06.2012

The Henry Kunkel Society Meeting "Immunological Memory in Health and Disease"
Berlin (DE) / 03-06.06.2012

Annual European Congress of Rheumatology "EULAR 2012"
Berlin (DE) / 06-09.06.2012

14th Annual Armenise-Harvard Foundation Symposium "Immunobiology of Microbial Host Interactions"
Borgo San Luigi, Siena (IT) / 10-13.06.2012

EAACI Congress 2012
Geneva (CH) / 16-20.06.2012

Trinity Biomedical Sciences Institute, Meeting on "IL-17 and Related Cytokines: Basic Biology to Clinical Applications"
Dublin (IR) / 18-21.06.2012

Conference on Cancer Immunotherapy "Modulating innate and adaptive immunity in cancer"
Paris (FR) / 29.06.2012

Cell Symposia "Human Immunity"
Lisbon (PG) / 19-21.08.2012

3rd European Congress of Immunology
Glasgow (UK) / 5-8.09.2012

Joint Cytokines 2012 and ICS/ISICR Annual meeting
Geneva (CH) / 11-14.09.2012

12th International Symposium on Dendritic Cells "New Paradigm of DC Science and Application"
Daegu (KO) / 7-11.10.2012

3rd International Conference on Regulatory T cells and Helper T Cell Subsets and Clinical Application in Human Diseases
Shanghai (CN) / 13-17.09.2013

EMBO Members Meeting 2012
Heidelberg (DE) / 24-26.10.2012

SIICA-SSAI Joint Workshop "Targeting Dendritic Cells For Immunity And Tolerance"
Ravello, Salerno (IT) / 8-9.11.2012

University of Ghent
Guest Seminar "T cell subsets in immunity, autoimmunity, and allergy"
Ghent (BE) / 16.11.2012

INGM Mini-symposium "T cell plasticity"
Milan (IT) / 30.01.2013

Cellular Therapy of Cancer Symposium
London (UK) / 27.02-02.03.2013

Marcus Thelen
Signal Transduction

Marcus Thelen

Marcus Thelen studied biochemistry at the University of Tübingen (DE) and 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 the Cohn/Steinman department. Biochemical aspects of cyotokine- and endotoxin-mediated phagocyte priming and cytoskeleton-mediated signal transduction were the topics of his studies. In 1992, he received a career development award (START) from the Swiss National Science Foundation and returned to the Theodor-Kocher-Institute at the University of Bern. He created his own research group working on molecular mechanisms of signal transduction in leukocytes, focusing on PI 3-kinase-dependent pathways and chemokine-mediated receptor activation. He obtained the *venia docendi* in 1994 and was awarded an honorary professorship in 2001 from the University of Bern. In 2000, he moved to Bellinzona and assisted in the opening of the IRB. Marcus Thelen heads since then the Laboratory of Signal Transduction.

Research Focus

The chemokine system is best known for its fundamental role in regulating leukocyte trafficking. Typically cell migration is governed by chemotactic cues consisting of chemokines which are produced by various cell types, such as endothelium, epithelium and stromal cells, and are often retained at their surface.
Within the chemokine system CXCL12 and its signaling receptor CXCR4 possess exceptional properties. Genetic deletion of either molecule leads to a similar lethal phenotype, which is exceptional as deletion of no other receptor or chemokine is fatal within the chemokine system. The phenotype is characterized by markedly impaired lymphopoiesis and myelopoiesis, imperfect vasculature, abnormal brain and heart development leading to perinatal death. These findings led to the assumption that CXCR4 and CXCL12 represent a monogamous receptor-chemokine pair. In addition expression of CXCR4 strongly correlates with the metastatic potential of diverse tumor cells. Among chemokine receptors, CXCR4 has unique signaling properties capable of promoting the sustained activation of intracellular signaling cascades, which is strictly dependent on the availability of extracellular CXCL12.
While most chemokine receptors follow a common paradigm of cell activation, more recently a small group of atypical chemokine receptors (ACKR) was described. ACKR share the heptahelical structure of rhodopsin-like receptors, but do not couple to G-proteins. Through their decoy function these receptors eliminate chemokines from the environment preventing inflammation or contributing to the resolution of inflammation. Accordingly, it was proposed that the function of this class of receptors is to regulate innate and adaptive immune responses by balancing the availability of chemokines for leukocyte trafficking. Our laboratory recently described CXCR7 as the unique decoy receptor for the homeostatic chemokine CXCL12, and revealed its expression on leukocytes. The receptor was shown to affect CXCR4 function by regulating the availability of the ligand and might also directly interact with CXCR4. Despite the lack of signaling through G-proteins, CXCR7 may use biased signaling though arrestins. The receptor plays a critical role in development, as targeted deletion in mice is lethal. The failure of coupling to G-proteins is consistent with the assumption that CXCR7 acts primarily as a scavenger. We provided formal evidence for such activity in mammalian cells.

Team

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

Molecular mechanisms of CXCR7sorting and potential signaling properties.

Marie-Luise Humpert and Marcus Thelen

– *CXCR7 in leukocytes*. We recently confirmed the expressions and function of CXCR7 on primary human B cells. In this project we confirmed the expressions and function of CXCR7 on primary human B cells. Complementary methods were used to provide unequivocal evidence. The expression of CXCR7 on non-hematopoietic cells and neoplasms is widely accepted, however, its expression on leukocytes was challenged. To solve the dissent we analyzed the expression of CXCR7 on human B cells. We validated the efficiency of different epitope-specific monoclonal antibodies to detect CXCR7 on transfected cells and primary human B cells. The specificity of the used antibodies was further confirmed by an experimentally independent double labeling approach. To this end CXCR7 was expressed in mammalian cells tagged at the N-terminus with an epitope suitable for covalent labeling. Moreover, examination of CXCR7-dependent scavenging of fluorescent-labeled CXCL12 revealed functional expression of the receptor on human and mouse B cells. Real-time PCR analysis of CXCR7 mRNA showed the presence of transcripts in human leukocytes. Finally two CXCR7-specific peptides were identified by mass spectrometry in immunoprecipitates from primary human B cells.

– *Functional role of CXCR7 in B cells*. The functional role of the atypical chemokine receptor CXCR7 was examined at late stages of B cell maturation, when B cells differentiate into antibody-secreting plasmablasts before they home to the bone marrow or to the mucosa and become long-lived plasma cells. We identified two populations of CXCR7^{+} cells in human tonsils, one being memory B cells the other being plasmablasts. The differential expression pattern on B cells, suggests a potential contribution of the scavenger receptor in the process of final B cell maturation. We found an inverse correlation of CXCR7 and CXCR5 cell surface levels. The findings suggest an important role of CXCR7 in regulating the migration of plasmablasts at late stages of B cell maturation.

– *Investigations of ligand-dependent and -independent receptor trafficking to elucidate the mechanism of chemokine scavenging*. Intracellular trafficking of CXCR7 is remarkably different from CXCR4. The temporal and molecular mechanisms of receptor sorting are not well characterized. With the aid of receptors fluorescently tagged at their N-terminus, fluorescent ligands, and fluorescent tagged markers of endosomal compartments the steps of cargo sorting are analyzed. Current investigations assign the C-terminus a critical role in receptor trafficking.

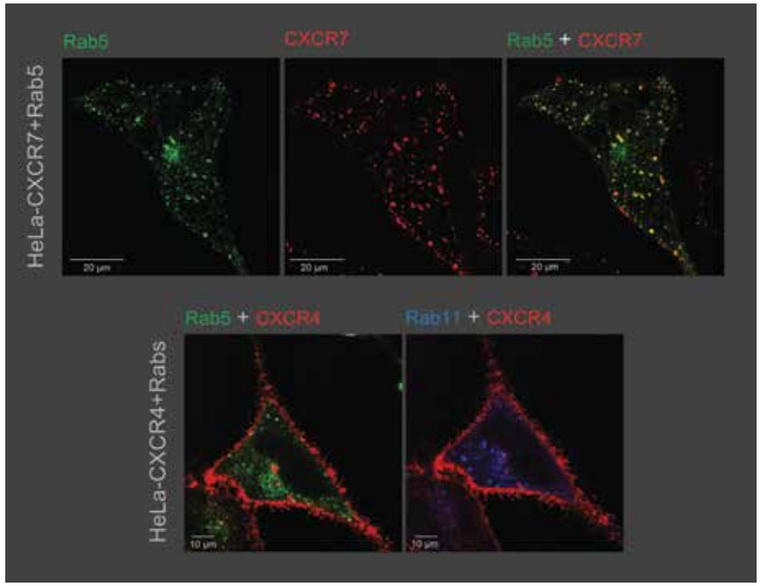

Figure 1
Ligand independent CXCR7 internalization.
Tagged CXCR7 (upper panels) and CXCR4 (lower panels) were labeled at 17°C with Atto-647-conjugated Co-enzyme A using phosphopantetheinyl transferase on the surface of HeLa cells expressing fluorescent protein fusion proteins of Rab5 (green) or Rab11 (blue). Cells were shifted to 37°C for 15 min an fixed. Images were taken with a confocal laser scan microscope (Leica SP5). CXCR7 is largely internalized after 20 min associating with the early endosome marker Rab5, whereas CXCR4 does not show spontaneous internalization.

Role of CXCR7 in cancer

Viola Puddinu and Marcus Thelen

The high incidence of aggressive lymphomas, which mostly originate from lymphocytes of the germinal center (GC), urges for additional therapeutic strategies, particularly when efficient treatments can fall into relapse. The best prognosis is given when the tumors are early diagnosed and confined to the primary site and the worst is when the cancer has metastasized. Metastasis formation requires migration of tumor cells from primary sites to niches where optimal growth and survival conditions are found. This migration requires the expression and function of chemotactic receptors.
Since the introduction of CXCR7 as chemokine receptor investigations describing the expression of the receptor on tumor cells is steadily growing. The reports focus mostly on tumors of mesenchymal cell origin. We have shown that CXCR7 is expressed in B cells and recent investigations have identified a subpopulation from human tonsils that expresses high levels of CXCR7. The high expression of CXCR7 during GC B cell differentiation correlates with the stage when cells, which continue to express CXCR4, are leaving the CXCL12-rich ambience of the GC. Several B cell lymphomas, in particular DLBCL, originate from distinct stages of B cell differentiation and continue to carry and exploit these characteristics as a program to survive and expand. In this project we investigate the expression of CXCR7 on DLBCL and Burkitt's lymphoma with respect to its effects on modulating CXCR4-dependent responses.

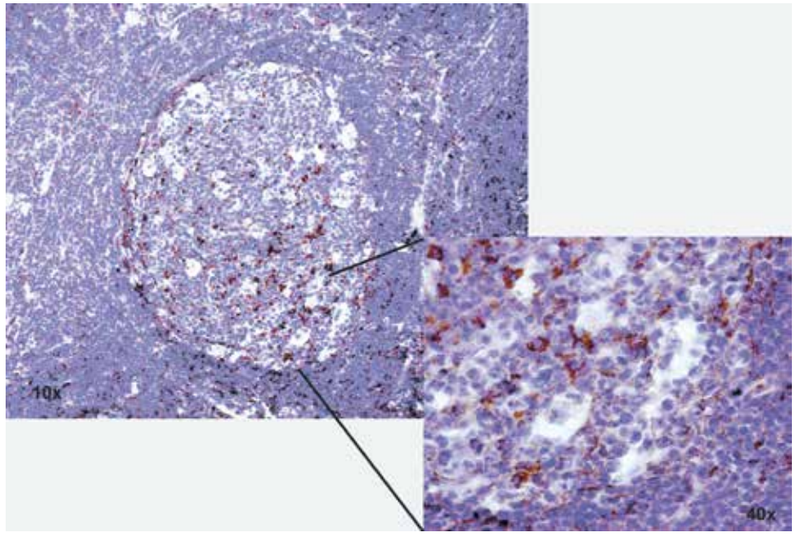

Figure 2
CXCR7 in Human Tonsil.
Paraffin section of human tonsil showing a B cell follicle. Red stain indicate CXCR7 positive cells in the follicle and surrounding area.

In vivo studies of CXCR7 function

Marcus Thelen

We recently identified CXCR7+ subpopulations in human tonsil B cells. Our findings are consistent with these cells being early, not fully differentiated plasma cells. For investigations of the potential role of CXCR7 in the B cell compartment we use mice with conditional deletion of CXCR7. To delineate a potential function of CXCR7 in the B cell compartment the animals are crossed with appropriate cre deleter mice. The lack of CXCR7 in the in the B cell compartment will be investigated with respect to the ability of the animals to mount an efficient immune response to different pathogens. At the same time the targeted B cells will express a fluorescent reporter enabling us to follow their localization by different fluorescence based techniques, such as FACS analysis, by confocal laser scan microscopy and multi-photon in vivo imaging.

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. Activation of the GTPases is catalyzed by specific GTP exchange factors (GEF). The phosphatidylinositol 3,4,5-trisphosphate (PIP3)-dependent exchanger 1 (P-Rex1) is assumed to be involved in G-protein coupled receptor (GPCR)-mediated Rac activation. P-Rex1 activity is stimulated by the PI 3-kinase product PIP3 and by the βγ subunits of heterotrimeric G-proteins, which are released upon activation of GPCRs. Consistent with the activation by these 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.
Investigations shall reveal the function of different P-Rex1 domains and their role in chemokine receptor-mediated signal transduction. In particular downstream of the chemokine receptor CCR2, a typical GPCR. To this end mouse bone-marrow derived hematopoietic precursor cells from animals lacking either the expression of P-Rex1 and/or P-rex2 or CCR2 are arrested by the conditional expression of HoxB8 and HoxA9. Various P-Rex1 mutants and variants of CCR2 tagged with epitopes suitable for fluorescent labeling are transduced into these cells and differentiated later into neutrophils and monocytes. Assessment of the chemotactic responsiveness allows to delineate the function of different P-Rex domains and the localization of the chemokine receptor CCR2 during migration.

Funding

Swiss National Science Foundation
Conventional and Atypical Chemokine Receptors: different mechanisms of function and common responses
3100A0-140704 / 2012-2015

Swiss Cancer League
Role of CXCR7 in B cell lymphoma
2012-2015

Gottfried und Julia Bangerter-Rhyner-Stiftung
Molecular mechanisms of CXCR7 sorting and potential signaling properties

Jost Reinhold Foundation
2010-2013

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

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Publications

CCR2 Acts as Scavenger for CCL2 during Monocyte Chemotaxis.
Volpe, S., E. Cameroni, B. Moepps, S. Thelen, T. Apuzzo and M. Thelen.
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HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4.
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J Exp Med. 2012; 209:551-563.

Complementary methods provide evidence for the expression of CXCR7 on human B cells.
Humpert, M. L., M. Tzouros, S. Thelen, A. Bignon, A. Levoye, F. Arenzana-Seisdedos, K. Balabanian, F. Bachelerie, H. Langen and M. Thelen.
Proteomics. 2012; 12:1938-1948.

Expression of CXCL12 receptors in B cells from Mexican Mestizos patients with systemic lupus erythematosus.
Biajoux, V., A. Bignon, C. Freitas, V. Martinez, M. Thelen, G. Lima, J. Jakez-Ocampo, D. Emilie, L. Llorente and K. Balabanian.
Journal of translational medicine. 2012; 10:251.

The guanine-nucleotide-exchange factor P-Rex1 is activated by protein phosphatase 1alpha.
Barber, M. A., A. Hendrickx, M. Beullens, H. Ceulemans, D. Oxley, S. Thelen, M. Thelen, M. Bollen and H. C. Welch.
Biochem J. 2012; 443:173-183.

Lectures and Seminars

Retreat of the Research Training Group University of Konstanz
Lecture: "Atypical chemokine receptors: CXCR7 expression and function"
Hornberg (DE) / 26-28. 02. 2012

Gordon Research Conference, Chemotactic Cytokines
Invited presentation: "Expression and function of CXCR7 in B lymphocytes"
Ciocco (IT) / 25.05.-01.06. 2012

University of Zurich
Seminar: "Atypical chemokine receptors: CXCR7 expression and function"
Institute of Virology, Zurich (CH) / 26.10.2012

EADV symposium
Lecture:"New from CXCR7"
Bellinzona (CH) / 08.11.2012

Multiple faces of the chemokine CXCL12
Invited presentation: "Why B cells (not) respond to CXCL12?"
Paris (FR) / 12. 12. 2012

Istituto Clinico Humanitas
Seminar:"Why B cells (not) respond to CXCL12?"
Milan (IT) / 14. 01. 2013

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), since 2000 she is group leader at the IRB, and vice-director since 2010. She is member of the Bologna Academy of Science since 2009. Mariagrazia Uguccioni's research has covered aspects of human haematology and immunology: chemokine expression and activities in normal and pathological conditions, leukocyte activation and traffic, natural chemokine antagonists and synergy-inducing chemokines. Recently, her group is focusing on chemokine activities in human inflammatory diseases, tumours, and infections and has identified a novel regulatory mechanism of leukocyte trafficking induced by synergy-inducing chemokines.

Research Focus

Our research interest remains focused on CHEMOKINE activities in physiology and pathology, with an emphasis on the mechanisms governing fine-tuning modulation of their expression and activity. Chemokines are secreted proteins and have emerged as key controllers of integrin function and cell locomotion. The effects of chemokines are mediated by seven transmembrane domain receptors coupled to GTP-binding proteins, which are differentially expressed in a wide range of cell types. The resulting combinatorial diversity in responsiveness to chemokines guarantees the proper tissue distribution of distinct leukocyte subsets under normal and inflammatory/pathological conditions. A vast range of *in situ* experiments, aimed at understanding which chemokines are produced in specific circumstances, has revealed that a variety of chemokines can be concomitantly produced at target sites of leukocyte trafficking and homing. This renders the chemokine system a good target for therapy, and has increased the search also by pharmaceutical companies for small molecule chemokine antagonists. While we understand the effects of different chemokines individually, much less is known about the potential consequences of the expression of multiple chemokines, cytokines, toll-like receptor ligands or other inflammatory molecules on leukocyte migration and function. Our group discovered the existence of additional features of chemokines: their ability to antagonize or enhance, as synergy-inducing chemokines, the activity of other chemokines. We have been the first to discover that the alarmin HMGB1 can enhance chemokine activities and contribute to the first phase of cell influx in injured tissues. Moreover, HMGB1 orchestrates cell migration and cytokine production by switching among mutually exclusive redox states. Reduced cysteines make HMGB1 a chemoattractant, a disulfide bond makes it a proinflammatory cytokine and further cysteine oxidation to sulfonates by reactive oxygen species abrogates both activities.

Team

Group Leader: Mariagrazia Uguccioni, MD > mariagrazia.uguccioni@irb.usi.ch
Members: Denise Bottinelli, PhD student – Valentina Cecchinato, PhD – Maria Gabriela Danelon, Technician – Camilla Marini, MSc – Lorenzo Raeli, PhD – Karolin Rommel, PhD student

Chemokines: Structure/Function Studies

Milena Schiraldi, Lorenzo Raeli, Camilla Marini, Gabriela Danelon and Mariagrazia Uguccioni*
**Former postdoc*

Chemokine structure/function studies led us to identify chemokines that can act as natural antagonists by preventing natural agonist binding and the subsequent activation of the receptor. Recently, we have described chemokines that can act in synergism with chemokine receptor agonists, forming heterocomplexes able to induce functional responses at lower agonist concentration. Several mechanisms have been proposed by us and other groups to provide an explanation for the synergy between chemokines: Dual receptor-mediated chemokine synergy and chemokine heterocomplexes (Figure 1).
After tissue damage, inflammatory cells infiltrate the tissue and release pro-inflammatory cytokines. HMGB1, a nuclear protein released by necrotic and severely stressed cells, promotes cytokine release via its interaction with the TLR4 receptor, and cell migration via an unknown mechanism. HMGB1-induced recruitment of inflammatory cells depends on CXCL12. HMGB1 and CXCL12 form a heterocomplex, which we characterized by nuclear magnetic resonance and surface plasmon resonance, that acts exclusively through CXCR4 and not through other HMGB1 receptors. FRET data show that the HMGB1/CXCL12 heterocomplex promotes different conformational rearrangements of CXCR4 from that of CXCL12 alone. Mononuclear cell recruitment in vivo into airpouches and injured muscles depends on the heterocomplex and is inhibited by AMD3100 and glycyrrhizin. Moreover, HMGB1 orchestrates cell migration and cytokine production by switching among mutually exclusive redox states. Reduced cysteines make HMGB1 a chemoattractant, whereas a disulfide bond makes it a proinflammatory cytokine and further cysteine oxidation to sulfonates by reactive oxygen species abrogates both activities.

* ***Schiraldi M. et al.***
J Exp Med. 2012, 209:551-563.
-Highlights, Nat. Rev. Immunol. 2012, 12:232.

* ***Venere E. et al.***
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* ***Gouwy M. et al.***
Immunol Lett. 2012, 145:10-14.

* ***Venereau E. et al.***
Mol Immunol. 2013, 55:76– 82.

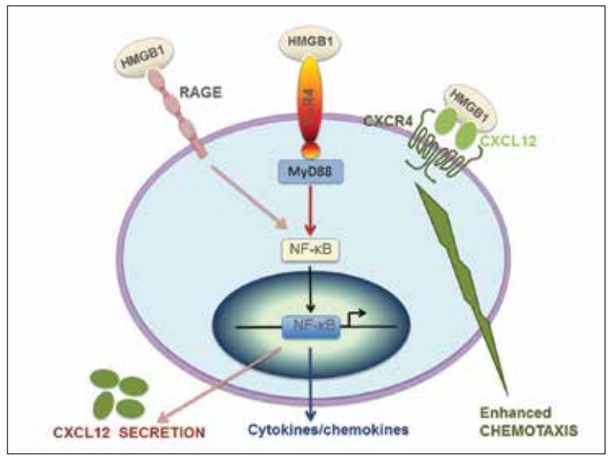

Figure 1
Model of action of HMGB1/CXCL12 synergism in leukocytes response.
HMGB1 binds CXCL12 and the heterocomplex activates CXCR4 and induces enhanced cell migration. HMGB1 binding to TLR4 and RAGE leads to the activation of NF-κB and the transcription of cytokine and chemokine genes. In particular, RAGE activation induces CXCL12 secretion.

Responses to chemokines in HIV/SIV infection

Valentina Cecchinato, Denise Bottinelli, Gabriela Danelon, 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 subset of effector T cells involved in the immune response against extracellular bacteria, has been described by Valentina 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 projects funded by the European Community and by the Swiss HIV Cohort Study, we are investigating the mechanisms that mediate $CCR6^+$/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 (Figure 2).

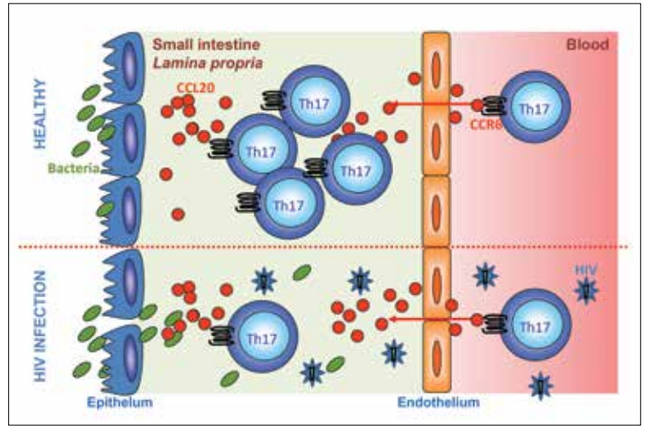

Figure 2
HIV reduces Th17 cells at mucosal sites.
We study the impact of chemokines and chemokine receptor expression on the migratory capacity of $CCR6^+$ cells.

Modulation of chemokine activity in spontaneous and therapy-induced breast cancer metastasis

Karolin Rommel and Mariagrazia Uguccioni

The chemokine receptor CXCR4 and its ligand CXCL12 are the most abundant receptor/ligand expressed in cancer. In a model of breast cancer, CXCR4 and CXCL12 were shown to be crucial for tumour cells migration and metastasis, and their blockade impairs metastasis formation. Physiologically, CXCL12 is one of the most abundant homeostatic chemokines, important for organogenesis, cell trafficking and bone marrow niches formation. CXCL12-induced migration has been broadly studied at molecular and cellular level in all cells bearing CXCR4. It has been demonstrated that high CXCL12 concentrations exert a repulsive effect on T cells. Following this study, it was discovered that repulsion of tumour Ag-specific T cells from a tumour expressing high levels of CXCL12 allows the tumour to evade immune control. Only recently, our laboratory and other colleagues discovered that chemokine activities can be modulated by non-ligand chemokines, concomitant activation of several chemokine receptors, or dimerization of chemokine receptors leading to synergism.
Chemotaxis of tumour and stromal cells in the tumour microenvironment is an essential component of tumour dissemination toward metastasis. Human and murine cancers possess a complex chemokine network that influences tumour cell behaviour, leukocyte infiltrate, and angiogenesis. The role of chemokines in cancer is modified in time and space by additional factors of the tumour microenvironment. This project aims at investigating the role of chemokines, in particular CXCL12, and HMGB1 for synergistic activities promoting breast cancer cell migration, invasion and metastasis.

HCMV glycoprotein complexes: characterization of their activity in modulating the migratory responses of cells of the immune system

Gabriela Danelon, Laurent Perez, and Mariagrazia Uguccioni

To understand the mechanisms governing the recruitment of immune cells induced by HCMV, we study the interaction of viral glycoprotein complexes, tested by the group of Antonio Lanzavecchia as candidate vaccines, with the human chemokine system. The gH/gL/UL128-130-131A pentamer complex, the gH/gL dimer and UL128 subunit were produced in transfected cells, purified and found to preserve all the conformational epitopes targeted by a panel of human neutralizing antibodies. We characterize the migratory capacity of cells transfected with human inflammatory and homeostatic chemokine receptors and of human monocytes and lymphocytes in response to selective stimuli and in the presence of the viral glycoprotein complexes in a soluble form.

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

Funding

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ADITEC: Advanced Immunization Technologies
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TIMER: Targeting Novel Mechanisms of Resolution in Inflammation
FP7 –281608 / 2012-2015

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

Swiss National Science Foundation
Impact of multiple chemokine expression in human disease
310003A- 143718 / 2013-2015

Swiss National Science Foundation – Swiss HIV Cohort Study
Impact of multiple chemokine expression in human disease
719 / 2013-2014

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

Novartis Stiftung für Medizinisch-Biologische Forschung
Dampening Inflammation in Autoimmunity by Targeting Chemokine synergy-inducing molecules
2012-2013

Jubilee Foundation Novartis
Fellowship to Lorenzo Raeli 2013

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Costantino Pitzalis
William Harvey Institute, London (UK)

Publications

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HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4.
Schiraldi, M., A. Raucci, L. M. Munoz, E. Livoti, B. Celona, E. Venereau, T. Apuzzo, F. De Marchis, M. Pedotti, A. Bachi, M. Thelen, L. Varani, M. Mellado, A. Proudfoot, M. E. Bianchi and M. Uguccioni.
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Immunol Lett. 2012; 145:62-67.

Lectures and Seminars

Istituto Clinico Humanitas
Synergism in chemokines: fine tuning modulation of biological
Milan (IT) / 02.03.2012

Gordon Research Conference
HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signalling via CXCR4
Lucca (Barga) (IT) / 31.05.2012

Luca Varani
Structural Biology

Luca Varani

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

Research Focus

Our group uses computational, biochemical and biophysical tools to determine the structure of proteins and characterize their interactions with other molecules, with particular attention to antibody-antigen interactions in infectious diseases.
Experimental techniques like nuclear magnetic resonance (NMR) and X-Ray crystallography have been traditionally used to investigate biomolecular structures at the atomic level. On the other hand, Computational Structural Biology is a novel, exciting field with very rapid development and high expectations for the near future. We can use computers to predict individual structures (modelling) and intermolecular complexes (docking) and the speed, precision and accuracy of these predictions is constantly increasing.
Computer predictions, however, are not always accurate, so it is important to experimentally validate them. What has largely been missing to achieve this goal is a concerted effort by different branches of the life sciences such as biology and informatics. Here we strive to merge biochemical data, experimental structural validation and computational docking in single workflow, and to apply it to biologically relevant cases such as the interactions between antibodies and pathogens or between cytokines, proteins responsible for biological signalling.

Team

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

Prediction and characterization of antibody-protein interactions in Dengue Virus

Luca Simonelli, Mattia Pedotti, Elsa Livoti and Luca Varani

Individuals that survive a viral infection have antibodies (Abs) capable of detecting and neutralizing subsequent attacks by the same virus. These Abs bind antigens (Ags), often viral proteins, through specific atomic interactions between the Ab and the region of the Ag that it recognizes (called epitope). 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 (since most vaccines generate an antibody response) or optimize the antibodies themselves for passive immunization strategies. 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. Schematically, we isolate Abs from the blood of human donors infected with a given virus; produce and purify human monoclonal antibodies (in collaboration with A. Lanzavecchia, IRB); characterize their immunological and biophysical properties; determine their epitope through NMR epitope mapping (Figure 1) 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.

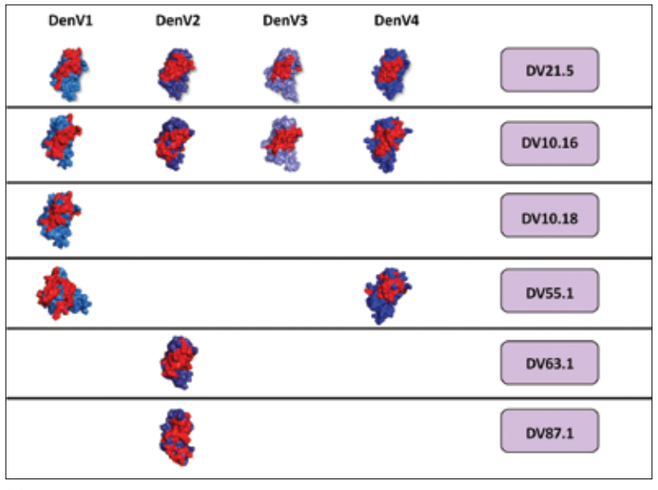

Figure 1
The epitope of several antibodies against Dengue was mapped with solution NMR spectroscopy.
Epitopes are shown in red over the surface representation of the so-called DIII, a part of the protein forming the surface of Dengue virus and the site recognized by most neutralizing antibodies against Dengue. Each line indicates a different antibody; the vertical columns correspond to the four main Dengue serotypes.

Dengue Virus: a case study

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

No cure or vaccine for DENV is currently available. The effort to find one has been hampered by the presence of four different dengue serotypes (DENV1–4) and by a poorly understood process almost unique in human medicine: antibody-dependent enhancement (ADE). Abs raised against a previous Dengue infection facilitate subsequent infection by a different serotype and lead to dengue hemorrhagic fever, an often lethal form of the disease. This feature complicates the task of finding a vaccine, since a vaccine that would not protect equally against all four serotypes would actually contribute to the emergence of dengue hemorrhagic fever. At the structural level, the most interesting region to study is the so-called Domain III of Dengue E protein (DIII), which forms the surface of the virus. DIII is the main target of neutralizing antibodies against DENV and it is relatively small, making it ideal for NMR and computational studies.

Our aim is to compare a large number of antibodies bound to DIII of the four Dengue serotypes, searching for correlations between immunological and structural trends and exploiting them to further our understanding of antibody-antigen interaction and ADE, as well as a basis for drug design and improved vaccine strategies. In a simplistic example, should we find that all Abs effective against DENV4 have a positive charge in a particular three-dimensional position, we would try to introduce such a charge in Abs lacking it, thus improving their characteristics. Conversely, should all effective Abs against a certain serotype recognize a particular epitope, then it is conceivable to prepare an antigen sharing the best epitopes of each serotype as a possible vaccination agent.

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

Rational antibody engineering to better neutralize Dengue

We determined the binding site of monoclonal antibody DV32.6 on DIII of all Dengue serotypes with NMR epitope mapping and used this information to guide and validate computational docking simulations yielding the three-dimensional structure of the antibody/antigen complexes. Visual analysis of such structures allowed us to design antibodies with altered binding sites that could: A) avoid binding to all serotypes. B) Bind only to one or two serotypes; eliminating unwanted cross-reactivity is a useful endeavor for therapeutic antibodies or when designing bio-recognition elements. C) Neutralize Dengue virus serotype 1 40 times more effectively than the original antibody or neutralize all serotypes between 10 and 17 fold better than the original molecule.

Overall, 18 out of 22 point mutations that we designed had the effect predicted by the computational models. 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).

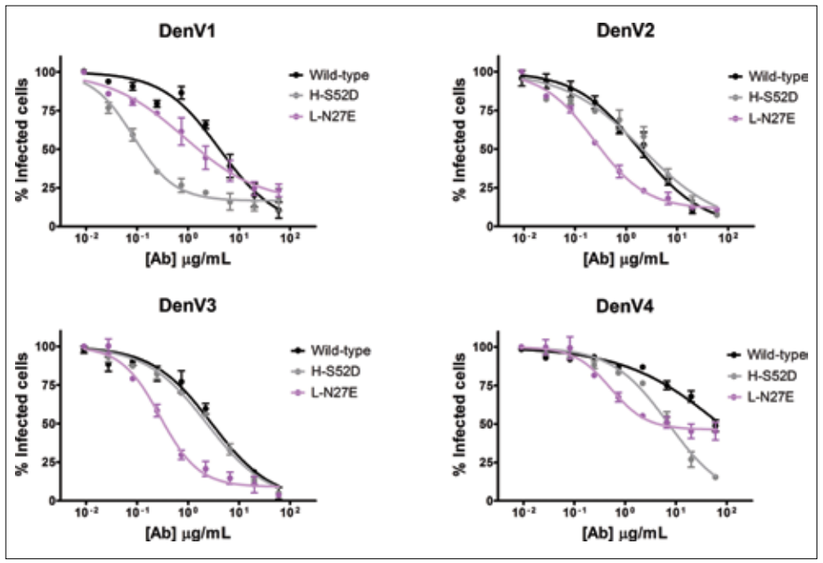

Figure 2
We designed two antibody mutants with the intent of improving its neutralization properties. H-S52D (gray) neutralizes DenV1 40 times more efficiently than the wild-type (black) and L-N27E (violet) is better than the wild-type in all serotypes, albeit to a lesser extent. Viral neutralization assays are shown; the amount of infected cells (y axis) decreases at increasing antibody concentration (x axis). In comparison to the wild-type, a lower concentration of mutants is required to neutralize the same amount of virus.

Structural Characterization of a potent neutralizer of Dengue Virus

DV87.1 is probably the most potent Dengue antibody described so far in the literature. It binds the surface protein of Dengue virus with nanomolar affinity and has an EC50 of 0.004µg/ml.

We used solution NMR spectroscopy to define its epitope on Dengue serotype 2 (figure 3) and then used this information to validate computational simulations aimed at obtaining the three-dimensional structure of the antibody in complex with its antigen. The antibody binds to an epitope not before described for other Dengue antibodies. Its position in the structure suggests that DV87.1 may neutralize Dengue Virus by blocking the conformational changes required for membrane fusion and infection of the host cells. Experiments are undergoing to obtain direct evidence on this hypothesis.

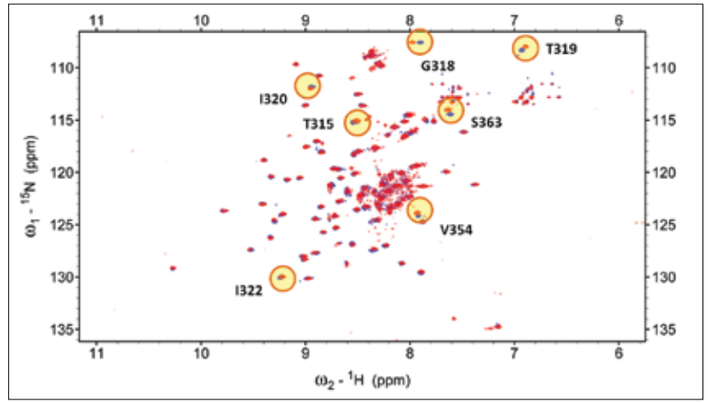

Figure 3
NMR spectrum of the Dengue antigen (DIII of DenV2) by itself (blue) and in complex with antibody DV87.1 (red).
In this type of experiment, each peak corresponds to an individual antigen residue. Differences in the position of the peaks between free and bound spectrum allow us to determine which residues are affected by antibody binding and are, thus, at the interface (epitope). Some of the residues whose signal differs between free and bound form are indicated in yellow.

RADAR: Rationally Designed Aquatic Receptors for the detection of organic pollutants

Mattia Pedotti and Luca Varani

The FP7 research consortium RADAR aims at developing label-free biosensor platforms for the monitoring of organic pollutants in the environment and for the surveillance of industrial production processes. The so-called Estrogen Disrupting Compounds (EDCs) are a class of pollutants capable of binding to human and animal estrogen receptors and disrupting their normal function. EDCs are known to be responsible for diseases ranging from cancer to sex changes in aquatic animals. Thousands of different EDCs exist, many of which still unknown. The concept behind the RADAR project is simple: EDCs exert their adverse function by binding to the Estrogen Receptor protein (ER). By using the ER as a bio-recognition element we can detect all compounds capable of binding to it and, therefore, potentially harmful.

Our role in the RADAR consortium is to design and produce a rationally modified ER that can 1) be easily attached to a sensor surface (via chemical tags); 2) be produced at low cost (E.Coli); 3) have an altered binding selectivity and increased affinity for selective organic compounds, so that a binding event would signal the presence of a particular class of compounds.

Structural analysis and computational docking allowed us to design a mutated ER capable of binding bisphenolic compounds with increased activity in comparison to the wild type protein. This mutated ER has been successfully produced, stabilized for a period of several months and attached to the surface of a label-free biosensor platform developed by the RADAR consortium.

Characterization of antibody-protein interactions in Diphtheria Toxin

Daniela Iannotta, 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, diphtheria toxin has been extensively characterized at the biochemical level and represents a good model for the study of antibody-toxin interactions. Curiously, however, no structural information on DT-antibody complexes is available so far.

The Lanzavecchia group has isolated a number of human monoclonal antibodies with 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 surface plasmon resonance (SPR) measurements. If not binding affinity, what are the determinants of efficient toxin neutralization?

DT is formed by three separated protein domains. ELISA, SPR and cross-competition experiments showed that the best neutralizing antibodies target different regions of the so-called receptor binding domain (figure 4). Perhaps unintuitively, however, we were able to show that the best antibodies do not inhibit interaction of DT with its cellular receptor. We are now trying to determine the mechanism of action of these antibodies through cellular and in vitro experiments. One possibility is that they might block the conformational change required for the activation of DT and subsequent toxicity.

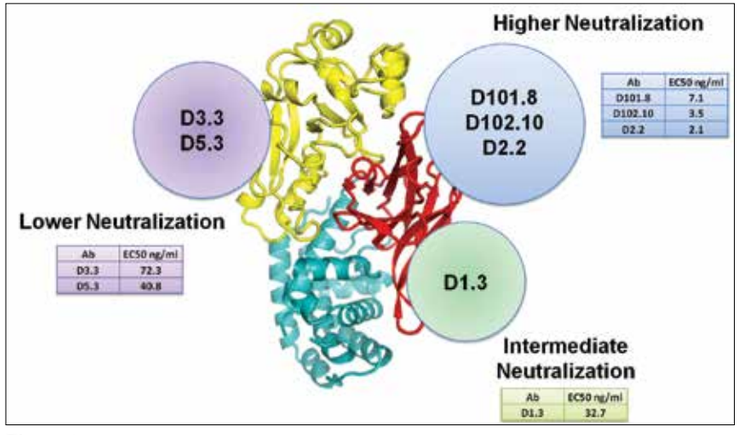

Figure 4

We were able to characterize three groups of human antibodies that bind Diphtheria Toxin (DT) with similar affinity (nanomolar) but have different neutralizing efficacy. Antibodies against the catalytic domain (yellow in the figure) are the worst neutralizer in our panel. Antibodies against the receptor binding domain (red in the figure) show intermediate to high neutralization according to their binding site on this domain.

Investigating the role of Interleukin 3 and TIM3 for the detection and elimination of Acute Mieloid Leukemia

Marco Bardelli, Luca Simonelli and Luca Varani

Many cancer cells show overexpression of particular proteins that can therefore be targeted by drugs or other therapeutic strategies, at least in theory. In acute myeloid leukemia (AML), a cancer with an high recurrence of relapses due to the presence of Leukemic Stem Cells that are not affected by normal chemotherapic drugs, these leukemic cells differ from normal ones for the strong overexpression of CD123 (IL3 Receptor Alpha) and other proteins such as TIM-3 and CD34 on the cell surface.

We are testing two approaches for selectively targeting AML stem cells with a bio-recognition element capable of discriminating them from normal, healthy cells. Such bio-recognition element could then be linked to either an engineered T-Cell chimeric antigen capable of killing leukemic cells (collaboration with Monza Hospital (IT)) or to nano-vectors capable of delivering drugs or irradiation/heating therapy directly to AML cells (collaboration with EU-Joint Research Center, Ispra (IT)). Previous literature has shown that the approach can succeed in leukemic forms different from AML.

The biggest problem in using overexpressed surface proteins as AML targets is that the bio-recognition element will also target some normal cells that express these proteins at low level. A therapy capable of killing AML cells would, therefore, also kill some normal healthy cells, with deleterious effects. We are attempting to overcome the problem with two complementary strategies. 1) We plan to generate a bi-specific antibody with one antigen binding site capable of recognizing CD123 and a second antigen binding site capable of recognizing TIM3. Both TIM3 and CD123 are expressed on the surface of normal cells but they are not both overexpressed on the same cell as it happens in AML. An antibody that would bind effectively only when it engages both TIM3 and CD123 may be, as a consequence, able to discriminate leukemic from healthy cells. 2) We plan to use either IL3 or an anti-CD123 antibody as bio-recognition element for CD123 (natural receptor alpha for IL3), which is overexpressed in AML cells. In order to avoid unwanted cross-reactivity with healthy cells, we want to investigate the effect of binding affinity on selectivity. Bio-recognition elements with lower binding affinity may engage cells in sufficient number only if their target (e.g. CD123) is abundantly overexpressed, as it is the case in AML cells. In order to tune the binding affinity we are using a combination of experimental methods (solution NMR mapping, mutagenesis, Surface Plasmon Resonance) and computational simulations (docking) to characterize the binding of IL3 and an anti-CD123 antibody to CD123. Visual analysis of the three dimensional structure of CD123 in complex with the aforementioned partners will allow us to design protein mutations with binding affinity lower than the original molecule but still sufficient for successful engagement of leukemic cells.

Funding

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RADAR: Rationally Designed Aquatic Receptors for the detection of organic pollutants
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Swiss National Science Foundation
Antibody-antigen interactions in Dengue virus
310030-138518-1 /2012-2015

SVRI
Studying the antibody response to primary and secondary Dengue infection
2012-2014

Synapsis Foundation
Antibodies as a tool to investigate prion protein toxicity
2013-2016

Collaborations

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BMRZ NMR Centre University of Frankfurt
Frankfurt, (DE)

European Union Joint Research Center
Ispra (IT)

Ana Paula Valente
University of Rio de Janeiro (BR)

Ettore Biagi
Tettamanti Research Centre, Monza Hospital (IT)

Nicholas Leulliot
Université Paris Descartes, Paris (FR)

Visiting Scientists

Alessandro Chiadò
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Publications

Gold nanoparticles downregulate interleukin-1beta-induced pro-inflammatory responses.
Sumbayev, V. V., I. M. Yasinska, C. P. Garcia, D. Gilliland, G. S. Lall, B. F. Gibbs, D. R. Bonsall, L. Varani, F. Rossi and L. Calzolai.
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J Exp Med. 2012; 209:1519-1528.

HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4.
Schiraldi, M., A. Raucci, L. M. Munoz, E. Livoti, B. Celona, E. Venereau, T. Apuzzo, F. De Marchis, M. Pedotti, A. Bachi, M. Thelen, L. Varani, M. Mellado, A. Proudfoot, M. E. Bianchi and M. Uguccioni.
J Exp Med. 2012; 209:551-563.

Lectures and Seminars

Congress: "Label-free technologies: advances and applications"
Amsterdam (NL) / 01-03.11.2012

European Conference on Computational Biology
Basel (CH) / 09-12.09.2012

Institute Pasteur
Paris (FR) / 06.04.2012

ASSOCIATE MEMBERS

SECTION 2

INDEX SECTION 2 – ASSOCIATE MEMBERS
121 Andrea Cavalli

Andrea Cavalli

Andrea Cavalli

Andrea Cavalli earned his degree in theoretical physics at the ETH in Zurich in 1995 and a PhD. in mathematics in 2001. After a period in the group of Amedeo Caflisch at the University of Zurich, in 2004 he joined the groups of Christopher Dobson and Michele Vendruscolo at the University of Cambridge, UK, with an Advanced Researcher Fellowship from the Swiss National Science Foundation. During this period of time, his work focused on the development of theoretical and computational methods for the determination of the structure of proteins from sparse experimental data. This line of research led to the development of the CHESHIRE method, which has enabled the first accurate determination of the native state of proteins using NMR chemical shifts (Cavalli et al., Proc Natl Acad Sci USA (2007), 104 9615-9620) and the structural characterization of the intermediate state of a protein (Neudecker et al., Science (2012), 336 (362-36). In December 2012, he joined the IRB as an Associated Member. His research is focused on the development of computational methods for the determination of the structure of folded and misfolded states of proteins from minimal sets of experimental data.

Research Focus

The accurate control in space and time of the assembly of the quaternary structure of multi-domain proteins is at the basis of essentially all biochemical reactions that take place in living organisms. Underlying these processes there are complex networks of interactions that take place over a wide range of length scales, from nanometres to micrometres, over time scales from nanoseconds to minutes and beyond, and involve molecular assemblies that vary in size from few atoms to hundreds of proteins.

In recent years increasingly detailed information about the structure and dynamics of proteins and protein complexes has been obtained by the development of innovative experimental techniques, in particular nuclear magnetic resonance (NMR) spectroscopy, and theoretical methods, notably molecular dynamics simulations.

The aim of the our research programme presented is to develop methods that will enable the combination of these two approaches by incorporating a wide range of different types of experimental data as restraints into computer simulations to provide an unprecedented description of the ensemble of structures that form the free energy landscape of proteins. A special emphasis will be devoted to understand the non-native interactions present in protein-folding intermediates that maybe implicated in amyloid fibril formation involved in neurodegenerative disorders, in particular Alzheimer's Disease.

Team

Associate Member: Andrea Cavalli, PhD > andrea.cavalli@irb.usi.ch

Determination of the free energy landscapes of proteins: application to Alzheimer's disease

Andrea Cavalli

A detailed description of folded and unfolded states of proteins is crucial for understanding many aspects of their life inside living cells, including the stability of the native state, the folding kinetics and the mechanism that may lead in some circumstances to misfolded states or amyloid fibers. Extended or partially extended states are, moreover, interesting in their own right as they are often involved in processes such a signal transduction, translocation across membranes and cell cycle regulation.

Most approaches used for protein structure determination use experimental data for the determination of a single structure and are, therefore, unable to describe the full free energy landscapes of proteins and fail to provide an accurate account of the dynamic aspect of proteins behavior.

Advanced free energy calculations by atomistic computer simulations provide a valuable complement to experimental methods. In recent years simulations are reaching time scales and molecular sizes that enable the calculation of the native structure of globular proteins up to 80 amino acids, on custom made clusters of computers.
Despite of these remarkable successes, several important problems remain unsolved. Fully atomistic simulations of membrane proteins, which are often difficult to crystallize and can not be studied by solution-state NMR, or of large molecular complexes formed by several proteins with an overall size of thousands atoms, are not yet possible because the time scales involved in the molecular rearrangements required to sample their energy landscape are to large, when compared with typical simulations times.
One way to make atomistic simulation more useful in practical problems is to use experimental information to guide simulations. As we have shown in our work this approach is used to routinely determine the native structure of protein domains up to 130 residues.
The novelty of the approach presented here is to combine experimental information such as NOEs, RDC and chemical shifts with advanced free energy calculation techniques, notably metadynamics, to provide a reconstruction of the relevant parts of the landscape of proteins.
Metadynamics has been successfully applied to a wide range of problems, ranging from chemistry to structure prediction. One of the major difficulties that metadynamics has to overcome is, however, the ad hoc choice of one or more suitable collective variables (CV) along which the free energy surface has to be calculated. Although some algorithms to solve this problem have been developed, we propose to use the agreement with experimental observable as a natural, unbiased set of CV.

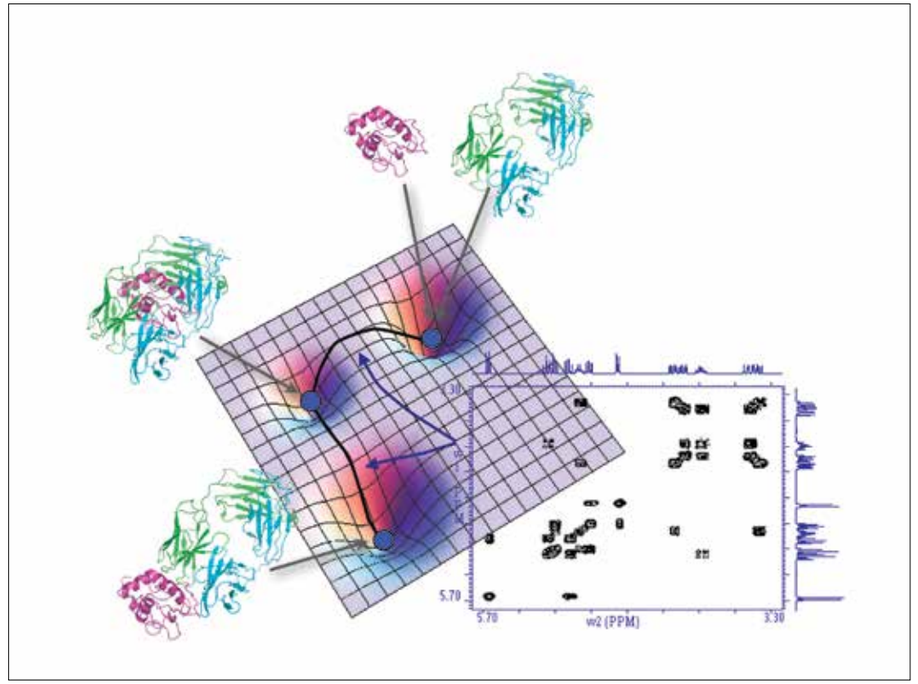

Figure 1
Free energy surface of an antibody-antigen complex formation process

Funding

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Determination of the free energy landscapes of proteins: application to Alzheimer's disease
2013-2014

Collaborations

Michele Vendruscolo
University of Cambridge (UK)

Publications

Molecular dynamics simulations with replica-averaged structural restraints generate structural ensembles according to the maximum entropy principle.
Cavalli, A., C. Camilloni and M. Vendruscolo.
J chem Phys. 2013; 138:094112.

Assessment of the use of NMR chemical shifts as replica-averaged structural restraints in molecular dynamics simulations to characterize the dynamics of proteins.
Camilloni, C., A. Cavalli and M. Vendruscolo.
J Phys Chem B. 2013; 117:1838-1843.

CORE FACILITIES

SECTION 3

INDEX SECTION 3 – CORE FACILITIES

129 Imaging Facility
130 Gene Expression and Protein Production Facility

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

Flow cytometry laboratory

The Flow Cytometry lab provides investigators with equipment and support for cell sorting (separation), acquisition, and analysis of flow cytometric data with a variety of state-of-the-art multicolor flow cytometry instruments. Cell sorting is performed via a FACSAria III sorter equipped with four lasers (488, 561, 640 and 405 nm excitation wavelengths) and 15 fluorescence channels detection. It can perform high speed sorting (up to 20,000 events/sec) with high-purity (up to 99%). The Flow cytometry lab is equipped with an advanced benchtop analyzers BDLSR Fortessa equipped with four lasers (488, 561, 640 and 405 nm excitation wavelengths) and 16 fluorescence channels. The lab also offers to researchers one FACSCantoII analyser (three lasers-eight colors), a FACSCanto I (two lasers-six colors), a FACSCalibur (two lasers-four colors) and a FACSArray plate based flow cytometer (two lasers-four channels). Both FACSCanto are equipped with HTS for high throughput screening for 96 and 384 wells plates. The lab is also equipped with a Flexmap 3D instrument for bead-based multiplexing for up to 500 analytes per well on a 96 or 384 well plate format. The staff provides cell sorting on BD FACSAria III, individual training on bench top analysers, maintenance of all instruments and assistance with experimental design, data analysis and troubleshooting.

Microscopy laboratory

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

– Confocal microscope. Leica sp5, equipped with new generation detectors (HyD detector) and high resolution objective (100x, NA 1.44)
– High-content imaging system. BD Pathway 855, wide-field or confocal automated microscope suitable

for screening. Equipped with Twister II Plate Handler (Caliper) allows to acquire and analyze up to 35 plates.

– Wide-field microscopes. Nikon Eclipse upright and inverted microscopes. A Zeiss axiovert 200 inverted microscope set for calcium measurement experiments.

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

Gene Expression and Protein Production Facility (GEPP)

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

PhD PROGRAMME

SECTION 4

INDEX SECTION 4 – PhD PROGRAMME

133 **Denise Bottinelli - Evaluation of the early changes occurring in the draining lymph node upon subcutaneous stimulation**
134 **Dora Pinto - Specificity, dynamics and novel properties of human plasma cells**
135 **Nicole Rusca - MiR-146a: a key microRNA involved in regulating mast cell survival and T lymphocyte differentiation**
136 **Zinaida Yudina - Structural basis of diphtheria toxin neutralization by human monoclonal antibodies**

Denise Bottinelli

Evaluation of the early changes occurring in the draining lymph node upon subcutaneous stimulation

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

***Reboldi A. et al.** **
Nat.Immunol. 2009, 10:514-23.

The response occurring in the draining lymph nodes (LN) early after peripheral injection of immunostimulatory molecules have been investigated by characterizing the cellular composition, and the profiles of cytokines and chemokines.
We observed that the peripheral administration of Complete Freund's adjuvant (CFA) in C57BL/6 mice induces very early, at 6 h, a potent influx of $CD11b^+$ cells in the LN, mainly consisting of neutrophils and monocytes. This observation prompted us to investigate the role played by the early recruited neutrophils in the T cell response generated. Interestingly, we observed a correlation between the influx of neutrophils and the increase in IL 23 and IL 1β, together with several inflammatory chemokines. Moreover, we confirmed the ability of murine neutrophils to express IL 23 upon stimulation with *Mycobacterium tuberculosis*. The increased expression of IL 23 we observed in the LN, is paralleled by a significant increased expression of the IL 23R. Our study of the different cell population, isolated from the LN, has identified $CD11c^+$ DCs as the principal cell type expressing IL 23R. Since the well documented involvement of different types of innate immune cells in the modulation of adaptive responses, we investigated the role of neutrophils in the generation of T helper responses upon CFA administration. We observed by in vivo priming a reduction in the percentage of IFN γ and CXCR3 expressing T cells upon depletion of neutrophils and a reduced activation state of these T cells. Altogether, we show that upon stimulation from the periphery, the draining lymph node undergo changes in cytokine/chemokine production leading to the recruitment of different leukocytes subpopulations. CFA induces a rapid influx of neutrophils which are responsible for the production of IL-23 that in turn influences the generation of T helper cells.

Dora Pinto

Specificity, dynamics and novel properties of human plasma cells

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

Plasma cells are terminally differentiated non-dividing cells of the B cell lineage that secrete antibodies at high rate. Although plasma cells can survive in appropriate bone marrow niches they die rapidly when cultured in suspension. In the first part of my thesis work we established culture conditions that allow to maintain plasma cells in long-term culture using IL-6 and mesenchymal stromal cells. These new methods were instrumental to study plasma cell biology and to screen plasma cell repertoires and led to two main projects. In the first project we studied the BCR expression in human plasma cells. We found that unlike IgG plasma cells which do not express surface BCR, IgA and IgM plasma cells express a functional BCR that when crossslinked can transduce signals leading to $Ca2^{+}$ flux and phosphorylation of Erk1/2 and Akt in a Syk-dependent fashion. Moreover only IgA plasma cells require BCR internalization for full induction of phosphorylation of Erk1/2 and Akt. Intriguingly, while IgM plasma cells express a full BCR comprising membrane IgM and the Igα:Igβ heterodimer, IgA plasma cells express IgA in the apparent absence of the heterodimer. Importantly these findings were obtained not only on *in vitro* generated or circulating plasma cells but also on terminally differentiated plasma cells isolated from the bone marrow and from colon lamina propria. Finally we found that BCR cross-linking on IgA plasma cells leads to CD44 up-regulation which may impact on their survival *in vivo*. Our findings demonstrate important differences in the biology of IgG, IgA and IgM plasma cells and also suggest that IgA plasma cells repertoire, especially at mucosal sites, may be modulated by the presence of the antigen.

In the second project we investigated the cellular basis for the maintenance of serum antibody levels. Using a high throughput culture system we studied the repertoire of human plasma cells that are found in blood 7 days following a booster immunization or in the steady state. In particular we analyzed the repertoire of recently generated plasma cells sorted as $HLADR^{+}CD62L^{+}$ and senescent or mobilized plasma cells sorted as $HLADR^{-}CD62L^{-}$. We found that in both cases recently generated plasma cells producing antibodies specific for unrelated recall antigens could be detected at low frequency. These findings support a dynamic model of serological memory where plasma cells are continually generated at low rate from memory B cells.

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

* ***Pinto D. et al.***
Blood 2013, 121:4110-4114.

Nicole Rusca

MiR-146a: a key microRNA involved in regulating mast cell survival and T lymphocyte differentiation

Supervisor: Silvia Monticelli // Co-referee: Jean Pieters
PhD in Cell Biology / Molecular Immunology, University of Basel

Mast cells are long-lived, tissue-resident cells of the innate immune system. Since the identification of mechanisms that regulate mast cell proliferation, survival and overall homeostasis in the tissues may have important implications for the treatment of mast cell-related diseases such as asthma, allergy and mastocytosis, we investigated the role of two activation-induced miRNAs, miR-221 and miR-146a in mast cell biology, we found that miR-221 has important roles in regulating multiple processes in differentiated primary mast cells, such as degranulation, adhesion, migration and cytokine production. Since miR-221 is expressed at basal level in mast cells but it is also inducible upon stimulation, we proposed a model in which miR-221 has a dual role in these cells: at resting state, it contributes to the regulation of the cell cycle and cytoskeleton, an effect probably common also to other cell types that express basal levels of this miRNA. However, in response to stimulation through IgE-antigen complexes, miR-221 effects are mast cell-specific and activation-dependent, contributing to the regulation of degranulation, cytokine production and cell adherence (Chapter 1).

Mice that lack the p50 subunit of NF-kB (p50KO) are unable to mount airway eosinophilic inflammation due to the inability to produce IL-4, IL-5 and IL-13, which play distinct roles in asthma pathogenesis, and to a defect in the polarization of Th2 lymphocytes. Since mast cells are master effector cells in allergic responses, we evaluated whether the asthma-resistant phenotype observed in p50KO mice could be partially due to a defect in mast cell development or function. While our data indicate that p50KO mast cells may only marginally contribute to the airway inflammation defect of p50KO mice through a slight impairment in cytokine production, p50KO mast cells showed a marked increase in their ability to survive in response to withdrawal of essential cytokines, which likely correlated with a strong increase in the percentage of mast cells that was observed in the tissues of p50KO animals. Such improved survival of mast cells lacking p50 was due to altered expression of several molecules involved in regulating cell survival and cell death, such as Bcl2, A1 and BAX. Importantly, we also found that miR-146a, a miRNA known to regulate NF-kB signalling, was not expressed in IgE- or LPS-stimulated p50KO mast cells, and that in the context of mast cell survival, miR-146a acted as a pro-apoptotic factor, identifying therefore a new molecular network that regulates mast cell survival in response to a variety of signals (Chapter 2).

In previous work from our lab also pointed toward a role for miR-146a in the differentiation and/or activation of murine CD4 T lymphocytes. We therefore continued investigating a possible role for this miRNA not only in regulating mast cell survival, but also in the differentiation and function of T cells. We found that miR-146a is expressed at high levels in the effector and effector-memory T cell compartment in both mouse and human, and we provide evidences that miR-146a may regulate T cell expansion upon activation and possibly also memory formation (Chapter 3). A review on the role of miR-146a in immunity and disease is provided in Chapter 4.

Overall our work demonstrates that miR-221 and miR-146a play a key role in regulating mast cell activation, function and survival, and that miR-146a also contributes to the extent of T lymphocyte activation. Finally, we provide novel insights on the role of miRNAs in regulating various functions of mast cells and T lymphocytes in the immune response, contributing to the groundwork for a further understanding of the molecular mechanisms that may lead to immune-related diseases such as asthma, allergy, altered inflammatory responses, and mastocytosis.

Mayoral R. J. et al. *
PLoS One 2011, 10:e26133.

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

Rusca N. et al. *
Mol Cell Biol. 2012, 32:4432-44.

Zinaida Yudina

Structural basis of diphtheria toxin neutralization by human monoclonal antibodies

Supervisor: Luca Varani // Co-referee: Alistair Mathie
Medway School of Pharmacy, University of Kent, United Kingdom

Diphtheria is a deadly disease caused by a bacterial toxin (DT). Although vaccination against it is widespread, its efficacy declines over the years and epidemics still arise. Currently equine antibodies (Abs) are used to treat diphtheria but they can cause adverse reactions. Although DT is a well-characterized model system to study the cellular activity of toxins, no structural information about the interaction between DT and antibodies is available.

My doctoral studies were devoted to the characterization of six anti-diphtheria toxin human monoclonal Abs. We revealed that the Abs can be divided into three main groups. Group 1 antibodies bind tightly to the catalytic domain (C domain) of DT and are not potent neutralizers of the toxin. Group 2 antibodies bind to the receptor-binding domain (R domain) of DT and show intermediate neutralization properties. Group 3 antibodies recognize the R domain in the so called “hinge loop” region and they are potent neutralizers of diphtheria. Since all antibodies have similarly strong binding affinity, this suggests that epitope localization is more important than binding affinity for effective neutralization of DT.Receptor binding assays show that group 1 antibodies cannot prevent DT binding to its cellular receptor. Group
2 and 3 antibodies prevent receptor binding by two different mechanisms. Group 2 binds nearby the receptor-binding site of DT, thus preventing receptor binding through steric hindrance. Group 3 antibodies bind close to the catalytic and receptor-binding domains.

Although the antibodies cannot directly obstruct the receptor-binding site, by keeping the catalytic and receptor-binding domain in close proximity they seem to prevent a conformational rearrangement of DT required for receptor binding. Remarkably, the latter antibodies are the most effective at neutralizing diphtheria.

*** *Simonelli L. et al.***
J Mol Biol. 2010, 396:1491-507.

PhD LECTURE COURSE & SEMINARS

SECTION 5

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139 PhD Lecture course 2012
140 Seminar Programme 2012

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

PhD Lecture Course 2012

Jos A.G. van Strijp
"Bacterial Immune Evasion"
University Medical Center Utrecht (NL) / 10.11.2011

Luigi Naldini
"Recent Advances in Hematopoietic Stem Cell Gene Therapy: from microRNA Regulation to Targeted Gene Transferd"
San Raffaele Telethon Institute for Gene Therapy, Milan (IT) / 08.02.2012

Hidde Ploegh
"Pathogens as unexpected allies of the biochemist"
Whitehead Institute for Biomedical Research, Cambridge, Massachusetts (US) / 02.03.2012

Yves Barral
"Aging at the cellular level: mechanisms and regulation"
Institute for Biochemistry, ETH, Zurich (CH) / 16.03.2012

Herman Waldmann
"Reprogramming the Immune System"
Sir William Dunn School of Pathology, University of Oxford (UK) / 26.03.2012

Charles Dinarello
"Blocking IL-1β in Autoinflammatory Diseases"
Division of Infectious Diseases, School of Medicine, University of Colorado Denver, Colorado (US) / 11.04.2012

Frank Slack
"MicroRNAs as targeted therapies and therapeutic targets in cancer"
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut, US / 10.05.2012

Ludger Klein
"Thymic epithelium and central T cell tolerance"
Institute for Immunology, Ludwig-Maximilians-Universität Munich (DE) / 24.05.2012

Marc K. Jenkins
"Origin of CD4+ memory T cells during bacterial infection"
Center of Immunology and Department of Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota (US) / 15.06.2012

Alessandro Sette
"The Study of Immune Reactivity as a Tool to Probe Host-Pathogen Interactions"
Center for Infectious Disease, La Jolla Institute for Allergy & Immunology, La Jolla, California (US) / 18.06.2012

Seminar Programme 2012

Maria Grazia Cusi
"Toscana virus: infection and pathogenesis"
Siena University School of Medicine, Siena (IT) / 16.01.2012

Santiago Fernandez Gonzalez
"Trafficking of B cell antigen in the lymph nodes"
Immune Disease Institute, Harvard Medical School, Boston (US) / 12.01.2012

Salomé LeibundGut
"T cell immunity to Candida albicans"
Microbiology Institute, ETH Zurich (CH) / 21.02.2012

D. Branch Moody
"CD1a as a target of Human Th22 Cells"
Brigham and Women's Hospital, Harvard Medical School, Boston (US) / 06.03.2012

Frank Heuts
"Mycobacterial infections in humanized mice"
Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, Stockholm (SE) / 12.03.2012

Stéphane Chevrier
"Identification and characterization of a new transcription factor involved in late B cell differentiation"
Walter & Eliza Hall, Institute of Medical Research, Parkville (AU) / 23.03.2012

John O'Shea
"Mechanisms underlying CD4 T cell plasticity"
NIAMS, National Institutes of Health, Bethesda, Maryland (US) / 26.03.2012

Daniela Latorre
"Immunomodulatory effects of bovine lactoferrin on antigen presenting cells"
Istituto Superiore di Sanità, Rome (IT) / 13.04.2012

Liam O'Mahony
"Immunoregulation by microbes and metabolites"
Swiss Institute of Allergy and Asthma Research (SIAF), Davos (CH) / 25.04.2012

Oreste Acuto
"MS-based proteomics to reveal T cell activation mechanisms"
Sir William Dunn School of Pathology, University of Oxford (UK) / 25.04.2012

Emilio Bossi
"Scientific integrity and scientific misconduct"
Committee of Scientific Integrity, Swiss Academies of Arts and Sciences (CH) / 07.05.2012

Costanza Casiraghi
"How common everyday pathogens like Epstein-Barr virus can induce autoimmunity"
Department of Microbiology and Immunology, The University of British Columbia, Vancouver, British Columbia (CA) / 08.05.2012

Emma Marie Caroline Slack
"The role of the liver in host-microbiota mutualism"
Institute of Microbiology, ETH Zürich (CH) / 19.06.2012

Sharon M. Blättler
"Yin Yang 1 Regulation of Insulin/IGF Signaling and Mitochondrial Function in Skeletal Muscle"
Dana-Farber Cancer Institute / Harvard Medical School, Boston, Massachusetts (US) / 13.07.2012

Kristine Germar
"Roles for T-cell factor 1 (Tcf-1) in lymphoid development"
Knapp Center for Lupus and Immunology Research, University of Chicago, Illinois (US) / 25.07.2012

Christian Ottensmeier
"Cancer Immunotherapy: Ready for prime time?"
Cancer Sciences Division and Department of Medical Oncology, Southampton University Hospitals, Southampton (UK) / 28.08.2012

Enrico Lugli
"Identification and characterization of T cells with stem cell-like properties in humans and nonhuman primates"
Vaccine Research Center, NIAID, NIH, Bethesda, Maryland (US) / 20.09.2012

Jérémie Goldstein
"CD122 signaling tunes short-lived Foxp3 expression in regulatory T cells"
Department of Medicine and Biology,
Université Pierre et Marie Curie, Paris (FR) / 26.09.2012

Carlo Ventura
"Lipogems®, New Device, Method and Human Fat Tissue Product Highly Enriched in Pericytes/Mesenchymal Stem Cells Ready for Autologous Use: Pluripotency optimization with chemistry and Physical Energy, and Implications for Regenerative Medicine"
Molecular Biology and Stem Cell Engineering, National Institute of Biostructures and Biosystems, University of Bologna (IT) / 11.10.2012

Curzio Rüegg
"Tumor-host interaction in tumor progression and therapy"
Department of Medicine, Faculty of Science, University of Fribourg (CH) / 12.10.2012

Roberto F. Speck
"Humanized mice: their value for studying HIV infection"
Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich (CH) / 25.10.2012

Markus Grütter
"New concepts in structural biology to facilitate protein production and crystallization"
Department of Biochemistry, University of Zurich, (CH) / 04.12.2012

Amalio Telenti
"Redefining components of the innate immunity"
Institute of Microbiology, University Hospital Center (CHUV), University of Lausanne (CH) / 06.12.2012

Gebhard F. X. Schertler
"Structures of active and inactive G-protein coupled receptors relevant to pharmacology and disease"
Biomolecular Research Laboratory, Paul Scherrer Institute, Villigen (CH) / 11.12.2012

PEOPLE & FINANCES

SECTION 6

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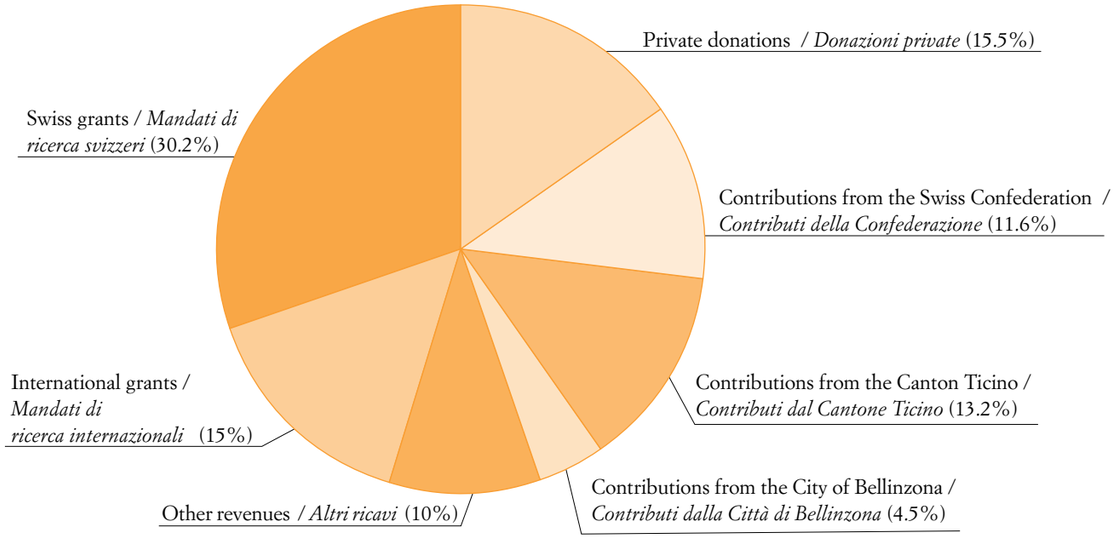
Financial Data 2012 (in Swiss Francs) / *Dati finanziari 2012 (in Franchi svizzeri)*

Overall, the year 2012 has seen a reduction in costs of 0.7 million if compared to 2011, with a significant increase in personnel costs (+ 1.0 million) and decrease in general costs (- 1.7 million). The reduction of the general costs is almost exclusively due to the reduction of investments (0.8 million in 2012, compared to 2.1 million in 2011).
In 2012, the funding for research activities reached 6.8 million, of which 3.9 million were for personnel costs. 33.2% of the research activities at the Institute were carried out with projects funded by foreign sources, in line with last year.

Complessivamente l'anno 2012 ha registrato una riduzione dei costi di 0,7 milioni rispetto al 2011, con un importante aumento dei costi del personale di 1,0 milioni e una riduzione di 1,7 milioni dei costi generali. La riduzione dei costi generali è determinata quasi esclusivamente dalla riduzione degli investimenti effettuati, 0,8 milioni nel 2012 rispetto a 2,1 milioni nel 2011.
L'attività di ricerca nel 2012 presenta un consuntivo di 6,8 milioni, di cui 3,9 milioni per costi del personale. Il 33,2 % dell'attività di ricerca è stata realizzata con progetti finanziati da fonti estere, in linea con l'anno precedente.

Funding by source 2012 / *Contributi per fonte 2012*



Balance Sheet as 31 of December 2012 (In Swiss Francs) / *Bilancio al 31 dicembre 2012 (in Franchi Svizzeri)*

ASSETS / *ATTIVO*		31.12.2012	31.12.2011
1.	Liquidity / *Liquidità*	11'007'624	9'420'304
2.	Receivables / *Crediti*	1'892'951	1'844'898
3.	Temporary Receivables / *Transitori attivi*	328'495	1'008'698
	Current Assets / *Attivo circolante*	13'229'070	12'273'900
4.	Participations / *Partecipazioni*	12'500	12'500
5.	Financial investments / *Investimenti finanziari*	2'000'000	1'959'663
6.	Buildings / *Immobili*	4'227'440	4'567'440
7.	Furnishing & Equipment / *Attrezzature*	460'000	880'000
	Fixed Assets / *Attivo fisso*	6'699'940	7'419'603
Total Assets / *Totale attivo*		19'929'010	19'693'503

LIABILITIES / *PASSIVO*		31.12.2012	31.12.2011
1.	Payables for goods and services / *Debiti per forniture e prestazioni*	747'795	676'066
2.	Accruals / *Accantonamenti e transitori passivi*	1'358'392	1'761'317
3.	Funds for Research Projects / *Fondi progetti di ricerca*	3'647'237	2'626'611
4.	Funds for Laboratories / *Fondi dei laboratori*	2'165'955	2'248'717
5.	Various Funds / *Fondi diversi*	1'819'268	2'361'514
	Current Liabilities / *Capitale estraneo a breve termine*	9'738'647	9'674'225
6.	Long Term Loans / *Prestiti a lungo termine*	2'800'000	2'800'000
	Long Term Liabilities / *Capitale estraneo a lungo termine*	2'800'000	2'800'000
7.	Capital Resources / *Capitale proprio*	7'219'278	7'175'810
8.	Annual Result / *Risultato d'esercizio*	171'085	43'468
	Equity of the Foundation / *Capitale della Fondazione*	7'390'363	7'219'278
Total Liabilities / *Totale passivo*		19'929'010	19'693'503

Profit and Loss Account for the year 2012 (In Swiss Francs) / *Conto economico esercizio 2012 (in Franchi svizzeri)*

COSTS / *COSTI*		2012	2011
1.	Personnel Costs / *Costi del personale*	7'016'418	5'996'271
2.	Consumables / *Fabbisogno medico*	2'208'097	2'169'745
3.	Maintenance of Buildings and Equipment / *Manutenzione immobili e attrezzature*	715'718	707'344
4.	Investments / *Investimenti*	828'259	2'113'084
5.	Amortizations / *Ammortamenti*	1'003'162	999'638
6.	Rent and Related Costs / *Affiti e altri costi dei locali*	1'301'722	1'342'705
7.	Administrative Costs and Various / *Costi generali amministrativi e diversi*	898'653	1'144'875
8.	Travels, Congresses and Guests / *Trasferte, congressi, viaggi e ospiti*	266'744	290'049
9.	Financial charges / *Oneri finanziari*	0	30'949
10.	Various Costs for Research / *Altri costi di ricerca*	714'699	719'344
Total Costs / *Totale costi*		14'953'472	15'514'004

REVENUES / *RICAVI*		2012	2011
1.	Contributions from the Confederation / *Contributi Confederazione*	1'755'000	1'729'000
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*	680'860	720'000
4.	Contributions from the Helmut Horten Foundation / *Contributi Fondazione Helmut Horten*	1'768'000	1'768'000
5.	Other Contributions / *Altri Contributi*	1'091'857	1'001'433
6.	Research Projects / *Progetti di ricerca*	6'833'797	7'562'556
7.	Other Revenues / *Altri ricavi*	995'043	776'483
Total Revenues / *Totale ricavi*		15'124'557	15'557'472

ANNUAL RESULT / RISULTATO D'ESERCIZIO	**171'085**	**43'468**

PUBLICATIONS

SECTION 7

INDEX SECTION 7 – PUBLICATIONS

153 Peer Reviewed Publications
157 Book Chapters

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

Peer Reviewed Publications from January 2012

304. **The guanine-nucleotide-exchange factor P-Rex1 is activated by protein phosphatase 1alpha.**
Barber, M. A., A. Hendrickx, M. Beullens, H. Ceulemans, D. Oxley, S. Thelen, M. Thelen, M. Bollen and H. C. Welch.
Biochem J. 2012; 443:173-183.

305. **Enhancement of chemokine function as an immunomodulatory strategy employed by human herpesviruses.**
Viejo-Borbolla, A., N. Martinez-Martin, H. J. Nel, P. Rueda, R. Martin, S. Blanco, F. Arenzana-Seisdedos, M. Thelen, P. G. Fallon and A. Alcami.
PLoS Pathog. 2012; 8:e1002497.

306. **HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4.**
Schiraldi, M., A. Raucci, L. M. Munoz, E. Livoti, B. Celona, E. Venereau, T. Apuzzo, F. De Marchis, M. Pedotti, A. Bachi, M. Thelen, L. Varani, M. Mellado, A. Proudfoot, M. E. Bianchi and M. Uguccioni.
J Exp Med. 2012; 209:551-563

307. **Functionally distinct subsets of human FOXP3^{+} Treg cells that phenotypically mirror effector Th cells.**
Duhen, T., R. Duhen, A. Lanzavecchia, F. Sallusto and D. J. Campbell.
Blood. 2012; 119:4430-4440.

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

309. **Systems Analysis of MVA-C Induced Immune Response Reveals Its Significance as a Vaccine Candidate against HIV/AIDS of Clade C.**
Gomez, C. E., B. Perdiguero, V. Jimenez, A. Filali-Mouhim, K. Ghneim, E. K. Haddad, E. D. Quakkerlaar, J. Delaloye, A. Harari, T. Roger, T. Dunhen, R. P. Sekaly, C. J. Melief, T. Calandra, F. Sallusto, A. Lanzavecchia, R. Wagner, G. Pantaleo and M. Esteban.
PLoS One. 2012; 7:e35485.

310. **Cutting Edge: LPS-Induced Emergency Myelopoiesis Depends on TLR4-Expressing Nonhematopoietic Cells.**
Boettcher, S., P. Ziegler, M. A. Schmid, H. Takizawa, N. van Rooijen, M. Kopf, M. Heikenwalder and M. G. Manz.
J Immunol. 2012; 188:5824-5828.

311. **Immunogenetic Mechanisms Driving Norovirus GII.4 Antigenic Variation.**
Lindesmith, L. C., M. Beltramello, E. F. Donaldson, D. Corti, J. Swanstrom, K. Debbink, A. Lanzavecchia and R. S. Baric.
PLoS Pathog. 2012; 8:e1002705.

312. **CCR2 Acts as Scavenger for CCL2 during Monocyte Chemotaxis.**
Volpe, S., E. Cameroni, B. Moepps, S. Thelen, T. Apuzzo and M. Thelen.
PLoS One. 2012; 7:e37208.

313. **Complementary methods provide evidence for the expression of CXCR7 on human B cells.**
Humpert, M. L., M. Tzouros, S. Thelen, A. Bignon, A. Levoye, F. Arenzana-Seisdedos, K. Balabanian, F. Bachelerie, H. Langen and M. Thelen.
Proteomics. 2012; 12:1938-1948.

314. **Role of the SEL1L:LC3-I Complex as an ERAD Tuning Receptor in the Mammalian ER.**
Bernasconi, R., C. Galli, J. Noack, S. Bianchi, C. A. de Haan, F. Reggiori and M. Molinari.
Mol Cell. 2012; 46:809-819.

315. **Purinergic P2X7 Receptor Drives T Cell Lineage Choice and Shapes Peripheral gammadelta Cells.**
Frascoli, M., J. Marcandalli, U. Schenk and F. Grassi.
J Immunol. 2012; 189:174-180.

316. **Role of lymphoid chemokines in the development of functional ectopic lymphoid structures in rheumatic autoimmune diseases.**
Corsiero, E., M. Bombardieri, A. Manzo, S. Bugatti, M. Uguccioni and C. Pitzalis.
Immunol Lett. 2012; 145:62-67.

317. **Possible mechanisms involved in chemokine synergy fine tuning the inflammatory response.**
Gouwy, M., M. Schiraldi, S. Struyf, J. Van Damme and M. Uguccioni.
Immunol Lett. 2012; 145:10-14.

318. **Chemokines in immunopathology: From dark sides to clinical translation.**
Mantovani, A., M. Locati and M. Uguccioni.
Immunol Lett. 2012; 145:1.

319. **Anti-CD3ε mAb improves thymic architecture and prevents autoimmune manifestations in a mouse model of Omenn syndrome: therapeutic implications.**
Marrella, V., P. L. Poliani, E. Fontana, A. Casati, V. Maina, B. Cassani, F. Ficara, M. Cominelli, F. Schena, M. Paulis, E. Traggiai, P. Vezzoni, F. Grassi and A. Villa.
Blood. 2012; 120:1005-1014.

320. **T-cell trafficking in the central nervous system.**
Sallusto, F., D. Impellizzieri, C. Basso, A. Laroni, A. Uccelli, A. Lanzavecchia and B. Engelhardt.
Immunol Rev. 2012; 248:216-227.

321. **T cell responses to known allergen proteins are differently polarized and account for a variable fraction of total response to allergen extracts.**
Oseroff, C., J. Sidney, R. Vita, V. Tripple, D. M. McKinney, S. Southwood, T. M. Brodie, F. Sallusto, H. Grey, R. Alam, D. Broide, J. A. Greenbaum, R. Kolla, B. Peters and A. Sette.
J Immunol. 2012; 189:1800-1811.

322. **Phenotypic Analysis and Isolation of Murine Hematopoietic Stem Cells and Lineage-committed Progenitors.**
Frascoli, M., M. Proietti and F. Grassi.
J Vis Exp. 2012; (65), e3736, doi:10.3791/3736.

323. **Negative regulators take center stage.**
Monticelli, S. and F. Sallusto.
Nat Immunol. 2012; 13:719-720.

324. **Antibodies against neutralization epitopes of human cytomegalovirus gH/gL/pUL128-130-131 complex and virus spreading may correlate with virus control in vivo.**
Lilleri, D., A. Kabanova, A. Lanzavecchia and G. Gerna.
J Clin Immunol. 2012; 32:1324-1331.

325. **Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release.**
Venereau, E., M. Casalgrandi, M. Schiraldi, D. J. Antoine, A. Cattaneo, F. De Marchis, J. Liu, A. Antonelli, A. Preti, L. Raeli, S. S. Shams, H. Yang, L. Varani, U. Andersson, K. J. Tracey, A. Bachi, M. Uguccioni and M. E. Bianchi.
J Exp Med. 2012; 209:1519-1528.

326. **Unconventional roles of nonlipidated LC3 in ERAD tuning and coronavirus infection.**
Bernasconi, R., J. Noack and M. Molinari.
Autophagy. 2012; 8:1534-1536.

327. **miR-146a and NF-kappaB1 Regulate Mast Cell Survival and T Lymphocyte Differentiation.**
Rusca, N., L. Deho, S. Montagner, C. E. Zielinski, A. Sica, F. Sallusto and S. Monticelli.
Mol Cell Biol. 2012; 32:4432-4444.

328. **Flagging and docking: dual roles for N-glycans in protein quality control and cellular proteostasis.**
Hebert, D. N. and M. Molinari.
Trends Biochem Sci. 2012; 37:404-410.

329. **Human Th17 subsets.**
Sallusto, F., C. E. Zielinski and A. Lanzavecchia.
Eur J Immunol. 2012; 42:2215-2220.

330. **IL-36 signaling amplifies Th1 responses by enhancing proliferation and Th1 polarization of naive CD4+ T cells.**
Vigne, S., G. Palmer, P. Martin, C. Lamacchia, D. Strebel, E. Rodriguez, M. L. Olleros, D. Vesin, I. Garcia, F. Ronchi, F. Sallusto, J. E. Sims and C. Gabay.
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331. **Pemphigus autoantibodies generated through somatic mutations target the desmoglein-3 cis-interface.**
Di Zenzo, G., G. Di Lullo, D. Corti, V. Calabresi, A. Sinistro, F. Vanzetta, B. Didona, G. Cianchini, M. Hertl, R. Eming, M. Amagai, B. Ohyama, T. Hashimoto, J. Sloostra, F. Sallusto, G. Zambruno and A. Lanzavecchia.
J Clin Invest. 2012; 122:3781-3790.

332. **Gold nanoparticles downregulate interleukin-1beta-induced pro-inflammatory responses.**
Sumbayev, V. V., I. M. Yasinska, C. P. Garcia, D. Gilliland, G. S. Lall, B. F. Gibbs, D. R. Bonsall, L. Varani, F. Rossi and L. Calzolai.
Small. 2013; 9:472-477.

333. **HMGB1 and leukocyte migration during trauma and sterile inflammation.**
Venereau, E., M. Schiraldi, M. Uguccioni and M. E. Bianchi.
Mol Immunol. 2012; 1:76-82.

334. **Expression of CXCL12 receptors in B cells from Mexican Mestizos patients with systemic lupus erythematosus.**
Biajoux, V., A. Bignon, C. Freitas, V. Martinez, M. Thelen, G. Lima, J. Jakez-Ocampo, D. Emilie, L. Llorente and K. Balabanian.
Journal of translational medicine. 2012; 10:251.

335. **Long-Term Heart Transplant Survival by Targeting the Ionotropic Purinergic Receptor P2X7.**
Vergani, A., S. Tezza, F. D'Addio, C. Fotino, K. Liu, M. Niewczas, R. Bassi, R. D. Molano, S. Kleffel, A. Petrelli, A. Soleti, E. Ammirati, M. Frigerio, G. Visner, F. Grassi, M. E. Ferrero, D. Corradi, R. Abdi, C. Ricordi, M. H. Sayegh, A. Pileggi and P. Fiorina.
Circulation. 2013; 127:463-475.

336. **Effect of the Purinergic Inhibitor Oxidized-ATP in a Model of Islet Allograft Rejection.**
Vergani, A., C. Fotino, F. D'Addio, S. Tezza, M. Podetta, F. Gatti, M. Chin, R. Bassi, R. D. Molano, D. Corradi, R. Gatti, M. E. Ferrero, A. Secchi, F. Grassi, C. Ricordi, M. H. Sayegh, P. Maffi, A. Pileggi and P. Fiorina.
Diabetes. 2013; 62:1665-75

337. **Broadly Neutralizing Antiviral Antibodies.**
Corti, D. and A. Lanzavecchia.
Annu Rev Immunol. 2013; 31:705-42.

338. **Memory T Cells in Latent Mycobacterium tuberculosis Infection Are Directed against Three Antigenic Islands and Largely Contained in a CXCR3(+)CCR6(+) Th1 Subset.**
Lindestam Arlehamn, C. S., A. Gerasimova, F. Mele, R. Henderson, J. Swann, J. A. Greenbaum, Y. Kim, J. Sidney, E. A. James, R. Taplitz, D. M. McKinney, W. W. Kwok, H. Grey, F. Sallusto, B. Peters and A. Sette.
PLoS Pathog. 2013; 9:e1003130.

339. **Rational Engineering of a Human Anti-Dengue Antibody through Experimentally Validated Computational Docking.**
Simonelli, L., M. Pedotti, M. Beltramello, E. Livoti, L. Calzolai, F. Sallusto, A. Lanzavecchia and L. Varani.
PLoS One. 2013; 8:e55561.

340. **Therapeutic Efficacy of Antibodies Lacking FcgammaR against Lethal Dengue Virus Infection Is Due to Neutralizing Potency and Blocking of Enhancing Antibodies.**
Williams, K. L., S. Sukupolvi-Petty, M. Beltramello, S. Johnson, F. Sallusto, A. Lanzavecchia, M. S. Diamond and E. Harris.
PLoS Pathog. 2013; 9:e1003157.

341. **Monoclonal IgG antibodies generated from joint-derived B cells of RA patients have a strong bias toward citrullinated autoantigen recognition.**
Amara, K., J. Steen, F. Murray, H. Morbach, B. M. Fernandez-Rodriguez, V. Joshua, M. Engstrom, O. Snir, L. Israelsson, A. I. Catrina, H. Wardemann, D. Corti, E. Meffre, L. Klareskog and V. Malmstrom.
J Exp Med. 2013; 210:445-455.

342. **Remote system for monitoring animal models with single-metabolite bio-nano-sensors.**
Carrara, S., L. Bolomey, C. Boero, A. Cavallini, E. Meurville, G. De Micheli, T. Rezzonico Jost, M. Proietti and F. Grassi.
IEEE Sensors Journal. 2013; 13:1018-1024.

343. **Persistent Antigen and Germinal Center B Cells Sustain T Follicular Helper Cell Responses and Phenotype.**
Baumjohann, D., S. Preite, A. Reboldi, F. Ronchi, K. M. Ansel, A. Lanzavecchia and F. Sallusto.
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344. **OMIP-018: Chemokine receptor expression on human T helper cells.**
Brodie, T., E. Brenna and F. Sallusto.
Cytometry A. 2013; in press.

345. **Dendritic cell homeostasis is maintained by nonhematopoietic and T-cell-produced Flt3-ligand in steady-state and during immune responses.**
Saito, Y., C. S. Boddupalli, C. Borsotti and M. G. Manz.
Eur J Immunol. 2013; in press.

346. **Specificity and Regulation of the Endoplasmic Reticulum-Associated Degradation Machinery.**
Merulla, J., E. Fasana, T. Solda and M. Molinari.
Traffic. 2013; in press.

347. **The role of miRNAs in mast cells and other innate immune cells.**
Montagner, S., E. M. Orlandi, S. Merante and S. Monticelli.
Immunol Rev. 2013; 253:12-24.

348. **A functional BCR in Human IgA and IgM Plasma Cells.**
Pinto, D., E. Montani, M. Bolli, G. Garavaglia, F. Sallusto, A. Lanzavecchia and D. Jarrossay.
Blood. 2013; in press.

350. **Fetal Human Cytomegalovirus Transmission Correlates with Delayed Maternal Antibodies to gH/gL/pUL128-130-131 Complex during Primary Infection.**
Lilleri, D., A. Kabanova, M. G. Revello, E. Percivalle, A. Sarasini, E. Genini, F. Sallusto, A. Lanzavecchia, D. Corti and G. Gerna.
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Book Chapters

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

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