Università della Svizzera italiana

Institute for Research in Biomedicine

Institute for Research in Biomedicine

Scientific Report
2013 - 2014

Institute for Research in Biomedicine

Scientific Report
2013 - 2014

This Scientific Report covers the 2013-2014 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 Gabriele Gendotti

President of the Foundation Council

Also in 2014, the Institute for Research in Biomedicine (IRB), a university-level institute affiliated to the USI, was able to consolidate its basic research activities in an international context. Its strengths include a leadership of international fame, the ability to obtain important competitive research grants, a network of excellent collaborations, and an environment that allows for the conducting of research with limited teaching activities.

Last year, the Director of the Institute, Prof. Antonio Lanzavecchia, and the Group Leader, Dr. Federica Sallusto, were named in the Reuters' Highly Cited Researchers 2014 List as two of the 3,215 most-influential researchers in the world, and were ranked amongst the 65 most-cited researchers in Switzerland and amongst the 87 immunologists with more impact on a world-wide scale.

Important grants were awarded that contribute decisively to the financing of scientific research at the Institute:
– BMFG - Bill & Melinda Gates Foundation- awarded twice to Prof. Antonio Lanzavecchia
– SNF to Dr. Silvia Monticelli
– SNF to Prof. Fabio Grassi
– SNF to Prof. Maurizio Molinari
– SNF Bonus of excellence to Prof. Antonio Lanzavecchia
– SNF R'Equip for the purchase of a Nuclear Magnetic Resonance (NMR) spectroscopy to Dr. Luca Varani
– SNF 120% support grant to Dr. Mariagrazia Uguccioni
– ERC Advanced Grant to Prof. Antonio Lanzavecchia.

In the course of the year, a new Center of Medical Immunology was established, directed by Dr. Federica Sallusto, which has been equipped with all the tools necessary to start research activity in a field with great development opportunities.

The Foundation Board also approved the message of March 12th, 2014 of the State Council for the establishment of a new Faculty of Biomedical Sciences and the creation of a new Master School in Human Medicine. The new Faculty of the USI, to which the IRB is affiliated, will strengthen the tie of the Institute to the academic world and encourage collaborations with the scientific contacts available in other Faculties. In addition, it would give the IRB the possibility to confer diplomas on its own, in particular the doctoral degree.

The quality of the research conducted at the IRB has been confirmed by the Scientific Advisory Board Members, who visited the Institute in March of 2015 and noted in their report: "[the IRB scientists] continue to contribute at the highest level internationally, but also made advances in technological approaches and successes in the field of health (impacting infectious diseases, vaccination, autoimmunity and other diseases). Evidence for success and fame of the IRB is shown by the strong collaborations with international researchers of high repute and the level of funding. The human monoclonal antibody technology approaches have the potential to address major unmet clinical needs, and continue to place Bellinzona at the forefront in the fight against numerous infectious and/or immune mediated diseases, with further important findings continuing to be made at the IRB. In Bellinzona, Humabs has potential to become an important and world competitive

prime successful biotech company, with significant impact on employment and technology in the Ticino area as already being witnessed".

In 2014, various conferences were organized by the IRB researchers, in particular:
- the SIICA - SSAI Joint Workshop by Santiago González with Matteo Iannacone and Jens Stein that took place from October 23rd-24th in Milan, in collaboration with the "Italian-Swiss Immunology Society Joint Workshop" entitled "Imaging the Immune System";
- the First European Chemokine and Cell Migration Conference by Marcus Thelen that took place from June 4th-7th in Villars-sur-Ollon.

The first "IRB Alumni Symposium" was also organized, which not only brought together researchers, Master and PhD students, as well as former employees and collaborators of the Institute, but also thanked and celebrated Prof. Giorgio Noseda for the great work done in favor of the IRB as founding member and first President of the Foundation.

Additional investments for the Gallera building were also made possible in order to improve the logistical structure and to best accommodate the researchers and PhD students.

The main objectives of the IRB Foundation Board remain those of consolidating the structure of the institute in order to achieve a critical mass that will guarantee productive stability and better opportunities of generational turnover at the level of the research groups, and to ensure the awarding of major grants to finance the different research activities in the future.

After the publication of the international competition for the design of the new headquarters of the IRB in the "ex-military camp" area, in which 34 teams of specialists from Switzerland and several European countries participated, and after a pre-qualification procedure, 10 proposals were chosen for final selection. The jury has in the meantime concluded the evaluation procedure of the eligible projects, proceeded in establishing a classification, and awarded prizes to the three most deserving projects. The Foundation Board has fully approved the conclusions of the jury and initiated the procedure to confer a mandate upon the winner of the design competition. The time schedule that foresees the completion of the building towards the end of 2020 has, for the time being, been respected.

On behalf of the entire Foundation Board and all the IRB researchers, we would like to renew our gratitude to our major sponsors, in particular to the Helmut Horten Foundation, the Ruth & Gustav Jacob Foundation, the Mäxi Foundation and the Gelu Foundation, as well as to our numerous sponsors and private donors that allow the Institute to continue to carry out its research in the best conditions, to train and promote young researchers while emphasizing, on a non-profit basis, the acquisition of new knowledge to proactively contribute towards generating the prerequisites for innovation, the improvement of the quality of life, and economic and social growth.

Atty. Gabriele Gendotti, *President of the IRB Foundation Board*

Bellinzona, July 2015

Foreword by
Antonio Lanzavecchia

Director of the IRB

The scientific report of the Institute for Research in Biomedicine (IRB) contains a succinct description of the research carried out in the course of 2013 and 2014. The main topics deal with the host defense against infectious agents and with the mechanisms of inflammatory and degenerative diseases.

The fruitful collaboration between researchers of the IRB and of Humabs BioMed, a spin-off company of the IRB, led to a *Nature* publication describing an antibody that neutralizes four different human and animal respiratory viruses. These results are encouraging for the development of new therapies and vaccines that can confer broad protection from diverse viruses, such as the Respiratory Syncytial Virus and the Metapneumovirus, which may be lethal in newborns and in immunocompromised patients. In another publication in *Nature*, the group of Antonio Lanzavecchia investigated the role of somatic mutations and showed that affinity maturation of influenza neutralizing antibodies occurs rapidly through a single mutation, which is followed by redundant mutations that diversify the antibody repertoire. Other studies from this group led to the dissection of mechanisms that can generate autoantibodies and mediate their involvement in pathology, and to the production of a promising vaccine against human cytomegalovirus.

In articles published in *The Journal of Experimental Medicine*, *PLoS Pathogens*, and *PNAS*, Federica Sallusto's group used a new method to study the repertoire of T lymphocytes against HIV-1 and *Mycobacterium tuberculosis* and in another study, published in *Immunity*, identified the mechanisms that promote differentiation and function of follicular helper T cells. Furthermore, by combining next generation sequencing with antigenic stimulation of selected T cell subsets, this group provided, for the first time, evidence for an extensive intraclonal diversification of CD4 memory T cells. The study, which is published in *Science*, supports a new model of T cell differentiation and has relevant implications for vaccination strategies.

The group of Fabio Grassi investigated the role of extracellular ATP in the regulation of intestinal immunity. In two articles published in *Cell Report* and *Immunity*, his group showed that the ATP receptor P2X7 controls the function of T helper cell-induced antibody response in the gut, thus promoting the mutualism between the host and microbiota. Moreover, in collaboration with the Federal Polytechnic School of Lausanne (EPFL), this group developed a system of nano-bio-sensors to monitor in real time the concentration of metabolites in a living organism. Furthermore, a collaboration with the group of Andrea Alimonti at IOR Bellinzona led to a joint publication in *Nature* on the role of myeloid cells in antagonizing the senescence process of cancer cells.

The group of Silvia Monticelli, in an article published in *Journal of Immunology*, identified the mechanisms that control the growth and function of mast cells, with implications for allergic diseases and mastocytosis. In another study, published in *Nature Communications*, this group identified the molecular mechanisms that modulate activation of human memory Th17 cells.

In an article published in the *European Journal of Immunology*, the laboratory of Marcus Thelen identified a new function for a chemokine receptor that regulates the migration of B lymphocytes and plasma cells. The group led by Mariagrazia Uguccioni studied new molecular interactions between chemokines and other

inflammatory mediators in autoimmune and infectious diseases. The group of Maurizio Molinari studied the mechanisms of protein folding and quality control that protect cells from the accumulation of misfolded proteins and may lead to a series of degenerative diseases.

The group led by Santiago Fernández González, has set up a two-photon microscope to visualize, in a living organism, the interactions between viruses and the cells of the immune system. This will be an asset to study the early events that underline the priming of the immune response.

IRB researchers are increasingly using computational approaches and combining them with experimental data to solve protein structure and understand protein-protein interactions. Using this approach the group of Luca Varani has engineered antibodies to Dengue virus with increased affinity and coverage of different serotypes. Andrea Cavalli, associate member of the IRB, has continued his successful collaboration with the Laboratory of Molecular Biology in Cambridge and has initiated a new line of research on protein aggregates.

The originality and the relevance of the research conducted at the IRB have been demonstrated by the numerous competitive funding granted to its researchers by the Swiss National Science Foundation, by the European Union and by the European Research Council. Presently, two IRB researchers hold the prestigious ERC Advanced Grant in recognition of the excellence and innovation of their research. The IRB researchers have received support also from the US National Health Institute, the Bill and Melinda Gates Foundation, and the Italian Cariplo Foundation.

Upon a generous donation by the Mäxi Foundation, the IRB had resources to set up the Gene Expression and Protein Production *(GEPP)* Facility and to recruit Laurent Perez, who is leading the facility and contributing to research in the field of vaccines. The *GEPP* team together with researchers in the Lanzavecchia's team developed a new vaccine against Human Cytomegalovirus (HCMV), which is being tested in preclinical models. The HCMV vaccine provides another example of the capacity of our institute to translate basic research into new therapies.

The IRB continues to play an important role in teaching. Our doctorate program has allowed 61 students to obtain their doctoral degree in research (PhD) at Swiss or European universities. Many of our students continue their careers with success in the academic world or in the biopharmaceutical industry. Thanks to the contribution from the Gustav & Ruth Jacob Foundation, the 32 doctoral students that today work at the IRB have access to a program of lessons and seminars held by international experts. Currently, the IRB collaborates with the Federal Polytechnic Schools of Zurich (ETHZ) and of Lausanne (EPFL), with the University of Zurich and with the Universities of Bern and Fribourg through the ProDoc program. In the future, the IRB will contribute to the teaching of immunology and biology in the framework of the "Master Medical School Ticino" of the Università della Svizzera italiana (USI). An opportunity to create a synergy between IRB and USI was offered by the Aldo e Cele Daccò Foundation, which has created at USI a position of Assistant Professor for Vittorio Limongelli, an expert in molecular dynamics.

On May 26th and 27th, 2014, the IRB organized its First Alumni Symposium at the Theater of Bellinzona. The Symposium was the opportunity to celebrate professor Giorgio Noseda and to thank him for the passion and the work done as Founder Member and First President of the IRB Foundation. During these years he not only helped IRB researchers by actively search for financial support, but also was among the promoters

of the collaboration agreement between the IRB and Humabs, which today guarantees a flow of income to the Institute and promotes translational research and employment in the biotechnology sector in Bellinzona. The Symposium was also the opportunity, the first on the eve of the 15th year of activity, to gather in Bellinzona, numerous researchers, students, and members of staff who have worked with us, helping to make the IRB today a world-class institute for biomedical research and for the formation of a the next generation of researchers.

Members of the IRB are often involved in the organization of scientific conferences and courses. Federica Sallusto organized, together with Hergen Spits and Dan J. Cua, the Keystone Symposium on "Emerging Cytokine Networks" in Vancouver, Canada on January 17-22, 2014; Santiago González together with Matteo Iannacone and Jens Stein co-organized the Italian-Swiss Immunological Society Joint Workshop entitled "Imaging the Immune System" which was held at the San Raffaele Scientific Institute in Milan, Italy on October 23-24, 2014; Marcus Thelen organized the first European Chemokine and Cell Migration Conference, which was held in Villars-sur-Ollon, Switzerland on June 4-7, 2015.

In closing, we would like to express our gratitude to all the current and past members of the Foundation Board for the success achieved in the search for funding and for the energy dedicated to the planning of the new building that will allow the IRB to expand and amplify its research areas. We are particularly grateful to our principal sponsors: the Helmut Horten Foundation, the City of Bellinzona, Canton Ticino and the Swiss Confederation. Our gratitude also goes to those who sustain us through donations and grants. We believe that the progresses and the achievements of the Institute will reward their dedication to the advancement of science.

Prof. Dr. med. Antonio Lanzavecchia,
Director of the IRB

Bellinzona, July 2015

RESEARCH GROUPS

SECTION 1

INDEX SECTION 1 – RESEARCH GROUPS

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 postdoctoral researcher in Michael Carroll's group at the Immune Disease Institute, Harvard Medical School, in Boston (USA). He has been awarded three EU Marie Curie Fellowships, one for his postgraduate studies in Denmark where he studied skin inflammation and the connection between innate and adaptive immune 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 recently obtained a third Marie Curie to initiate his own independent group at the Institute for Research in Biomedicine in Bellinzona (IRB). He has published several papers about antigen trafficking, memory B cells, and the regulation of the immune system in high-impact journals. During his work at Harvard he studied the transport mechanism of 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 viruses through vaccination and were published in *Nature Immunology*. In November 2012 he joined the IRB as a group leader studying pathogen-host interactions. He has been selected by the Swiss National Science Foundation to join the competitive *Ambizione* program.

Research Focus

The primary focus of the laboratory is to study the interactions between pathogen and host. Research interests include the innate and adaptive immune responses to respiratory pathogens, and the mechanisms by which such viruses and bacteria fight the host's immune system. The initial response of the body to infection involves a series of events characterised by the rapid upregulation and recruitment of effector molecules and cells, which facilitate the elimination of the pathogen and the restoration of homeostasis. However, this response is not unidirectional. Pathogens have developed complex strategies to initially challenge the immune system of the host but also to resist its counter-attack. A better understanding of the virulence mechanisms of pathogens will contribute to the development of new strategies to fight infection. The mechanisms involved in the initial host response against infection will also be studied. The combination of the two previous perspectives will contribute to the better understanding of the immune response to disease challenges, allowing the design of more effective ways of enhancing the host immune response.

We are currently using state-of-the-art imaging techniques, including 2-photon intravital microscopy and confocal microscopy, to address some of the aforementioned questions. These techniques enable us to study the interactions between the pathogen and the host in a new dimension, examining cell-to-cell and microbe-to-cell interactions in real time. In addition, we use some classic imaging techniques, electron and scanning microscopy, to increase image resolution in order to obtain additional structural information from infected tissues or cells.

Team

Group Leader: Santiago F. González, PhD, PhD > santiago.gonzalez@irb.usi.ch

Members: Nikolaos Chatziandreou, PhD - Yagmur Farsakoglu, PhD student - Miguel Palomino, PhD student - Diego Pizzagalli, PhD student - Rocco D'Antuono, Imaging Specialist.

Characterisation of the role of natural killer cells in the immune response against influenza virus

Yagmur Farsakoglu and Santiago F. González

Natural killer cells (NK) play a crucial role in eliminating virus-infected as well as stressed and cancerous cells. It has been previously shown that NK cells require priming by other immune cells, such as macrophages and dendritic cells, in order to function optimally. In addition, different studies have also demonstrated that NK cell activation is required for T cell priming in lymph nodes (LN). The aim of this study is to investigate the localisation and response of NK cells to influenza vaccine in the popliteal lymph node after subcutaneous injection by the use of state-of-the-art 2-photon intravital microscopy. Moreover, we will evaluate the interaction of NK cells with some of the major immune cell populations in the LN, both at steady state and various time points post vaccination, to elucidate the significance of their interactions in the response to influenza vaccine. Flow cytometric analysis and intravital imaging indicated that immunisation results in a fast recruitment of activated NK cells to the draining LN, which reaches a peak at 12 hours post vaccination. At that time, NK cells show stable and prolonged interactions with activated CD169+ macrophages both in the medullary and subcapsular sinus regions of LN. Furthermore, we could observe a clear correlation between NK cell recruitment and the presence of retained vaccine. In this study we will characterise the mechanism by which the activation of the LN macrophages contributes to the recruitment of NK cells and the role of macrophage-NK cell interactions in the development of a protective adaptive response against influenza vaccine.

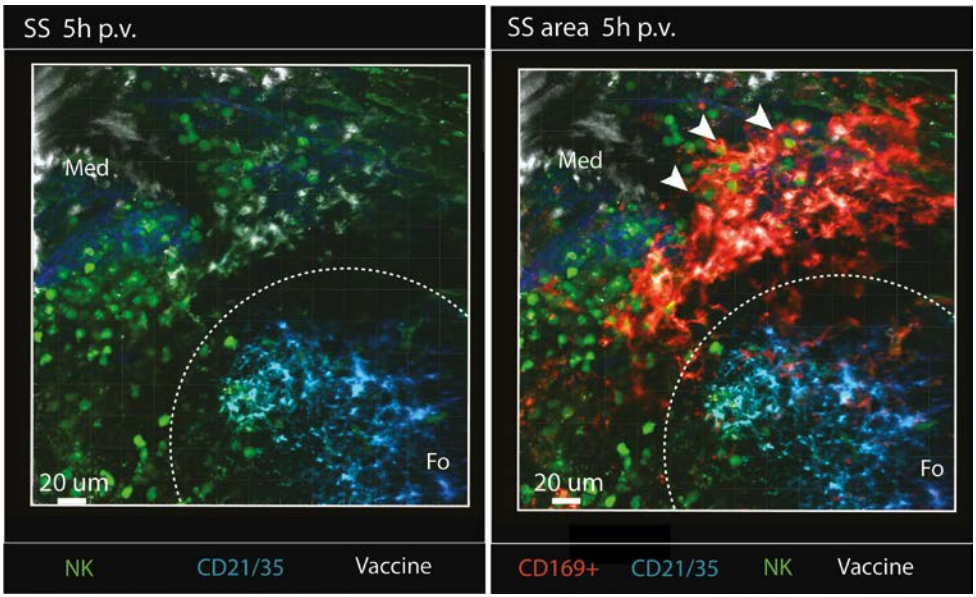


Figure 1
2-Photon intravital micrograph showing NK cells (green) interacting with CD169+ macrophages (red) in the popliteal lymph node.

Development of new algorithms and methods for identification and tracking of leukocytes in time-lapse microscopy

Diego Pizzagalli and Santiago F. González

The immune system protects us from pathogens and cancer through distinct cellular and molecular interactions. White blood cells, also referred to as leukocytes, are the key effectors of the immune system. Leukocytes can become highly motile in response to stimuli, such as inflammatory chemokines or during the execution of internal programs such as apoptosis. The analysis of movement patterns is then a powerful tool to estimate and characterise the behaviour of leukocytes.
In the last decades, advances in time-lapse imaging have given rise to techniques such as two-photon intravital microscopy *(2P-IVM)* that allows the visualisation of immune cell interactions *in vivo*. However, tracking the movement of leukocytes is far from automatic. Firstly, the identification of the shape of leukocytes is difficult, since cells cluster and boundaries are blurred. Secondly, cells may have different motion patterns. Last, the sampling rate is usually kept to a minimum in order to avoid phototoxicity and reduce the size of acquisition files. For these reasons, the currently available software and methods require that the user sets non-biological parameters and manually corrects tracks, which is time-consuming and introduces bias. We propose to develop specialised software for automatic analysis of leukocytes motion, which aims to improve tracking accuracy while keeping its execution feasible on commercial workstations. The proposed software and methods exploit spatial-temporal features in an inverse problem framework, by detecting forward models of leukocyte motion in 2P-IVM data. This will result in intrinsic segmentation and tracking. Furthermore, our method will be designed to be executable on massively parallel computing architectures provided by modern GPUs and will be implemented as a plugin for imaging applications such as Bitplane Imaris and ImageJ.

This work is done in collaboration with Marcus Thelen (IRB) and Rolf Krause, Institute of Computational Science, University of Lugano (CH).

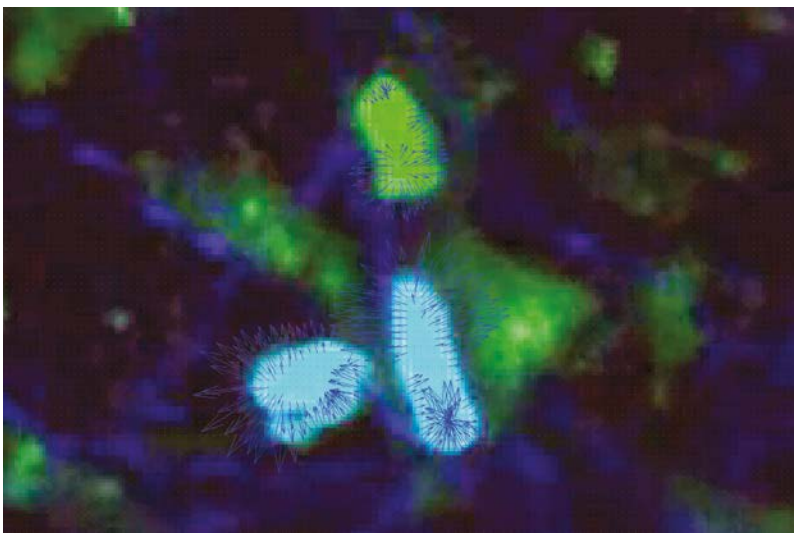


Figure 2
Optical flow computation on neutrophils (blue) moving towards a dendritic cell (green).

Intravital imaging of polymicrobial respiratory diseases

Miguel Palomino and Santiago F. González

Respiratory infections are one of the leading causes of disease and mortality worldwide. Amongst the major respiratory pathogens, influenza virus and *Streptococcus pneumoniae*, have an impact on public health that incurs very high healthcare expenses every year. It has been demonstrated that, in some cases, co-infection with a particular combination of pathogens results in a more severe clinical outcome compared with infection with either of the pathogens alone. Nevertheless, the mechanisms by which influenza co-infection may facilitate pneumococcal secondary infections remain unclear. This project regards the investigation of the host-pathogen interactions upon co-infection with influenza virus and *S. pneumoniae*, using state-of-the-art molecular techniques (microbiome analysis, microarray analysis) and intravital microscopy methods (2-photon intravital microscopy) in the mouse model (Figure 3). To this end, two aims are proposed. Firstly, we will characterise the molecular mechanisms that lead to the transition from normal microbiota to an infected state in the upper respiratory tract, taking into account the host-pathogen interactions that lead to infection. We will develop a predictive model of how the transcriptome and microbiome signature of the host may be affected in response to influenza infection so that we can assess whether this perturbation could lead to a secondary pneumococcal infection.

In the second aim we will examine *in vivo* the effects that the molecular mechanisms identified in the previous aim have in the establishment of a pneumococcal secondary infection. We will develop a 2-photon intravital microscopy model of the mouse trachea and different fluorescent strains of influenza and pneumococcus will be produced to visualise their interaction with the host tissue. This technique will allow us to monitor the dynamics of co-infection in real time.

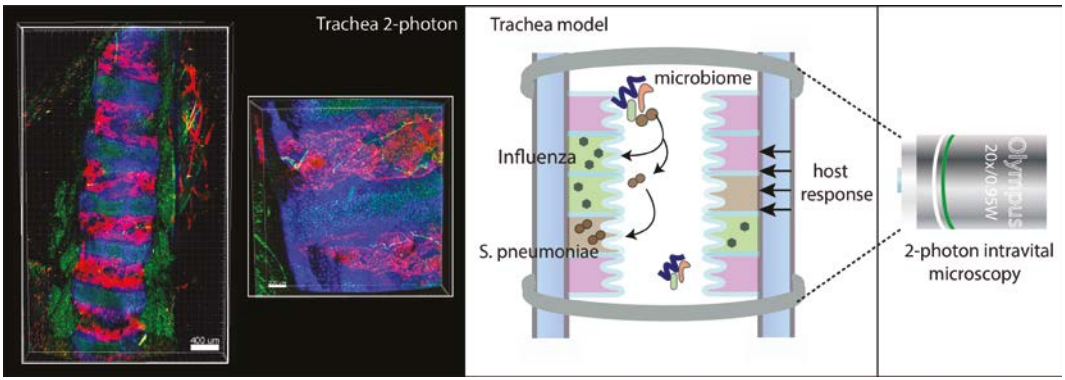


Figure 3
Schematic representation of the trachea of a mouse infected by influenza and pneumococcus.

Role of neutrophils in the immune response to virus and bacteria

Nikolaos Chatziandreou and Santiago F. González

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 microbicidal peptides directed towards the pathogen. In addition, some studies have suggested a regulatory role, affecting CD8 T cell priming or their interaction with dendritic cells (DC). Interestingly, a recent study has observed a competitive role for antigen between neutrophils and antigen-presenting cells (macrophages and DC) in the lymph node. The authors conclude that neutrophils have an important role affecting CD4 T cell and B cell responses to three protein antigens: hen egg white lysozyme, ovalbumin and listeriolysin. In this study we observed that the injection of influenza virus was accompanied by a significant increase in the number of infiltrated neutrophils in the subcapsular sinus area of the draining lymph node. Interestingly, we observed that the infiltrated neutrophils were located in the proximity of subcapsular sinus macrophages. The aim of this project is to characterise 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 these cells in the initiation of the protective response against different pathogens. To achieve this goal we will use 2-photon intravital microscopy.

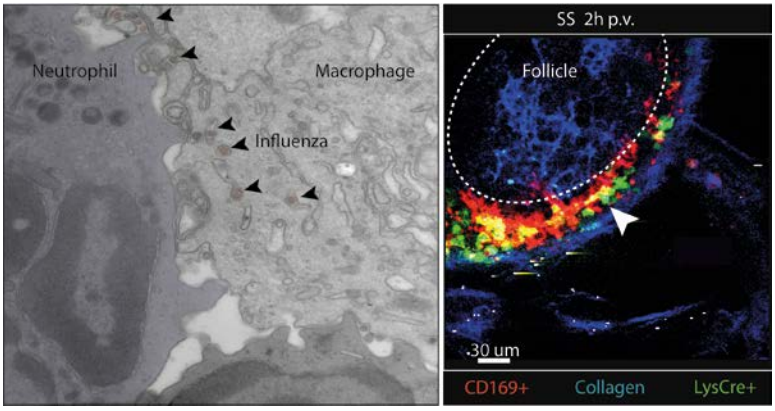


Figure 4
Electron micrograph (left) and 2-photon snapshot (right) showing the macrophage-neutrophil interaction in the lymph node.

Lymph node macrophages as initiators of the immune response

Nikolaos Chatziandreou and Santiago F. González

Innate immune cell responses to influenza vaccine play a key role in the host's defence against the virus. Lymph nodes are increasingly the focus of investigation for innate immune cell interactions after vaccination. Our previous studies of influenza vaccination in the mouse model have shown that the macrophages that line the subcapsular sinus of the lymph node (SSM) capture inactivated influenza virus, affecting antigen spread in the host. We have also observed that, following vaccination, SSM undergo a cell death program. The aim of this project is to elucidate the mechanism of SSM death after vaccine administration and determine whether it affects the host's antibody response to the vaccine. To this end, we are examining innate immune cells and their interactions in the mouse lymph node after vaccination, employing flow cytometry, 2-photon intravital microscopy and cytokine profiling. Our data have confirmed that SSM are eliminated in a dose-dependent manner as early as 3 hours after vaccine administration, through a mechanism that is MyD88-mediated. Experiments using ASC (apoptosis-associated speck-like protein containing a CARD) and caspase-1 knockouts failed to identify these inflammasome pathway components as participants in the observed cell death program, necessitating further studies to characterise the signalling pathways involved. In addition to SSM, the number of lymph node medullary macrophages (MM) is also affected after vaccination. In the presence of lower amounts of antigen plus adjuvant, MM were eliminated, contrary to SSM that remained present, indicating that the mechanisms that determine SSM and MM survival are different. Markedly, the same treatment led to an increase in IL-1alpha and CXCL13 levels in lymph isolated from the lymph node proximal to the injection site. This was accompanied by a higher number of recruited immune cells, indicating that the observed macrophage death is part of a mechanism for antigen presentation that does not compromise the ability to contain secondary infections in the lymph node.

Our study has identified innate immune cell responses to influenza vaccine in the lymph node, focusing on SSM death as a central event that affects the ability of the host to capture and contain antigen. The mechanism of SSM and MM death and the role of this phenotype in antibody responses remain to be elucidated, in order to understand the processes that occur after vaccine administration. This will contribute to the improvement of influenza vaccine design.

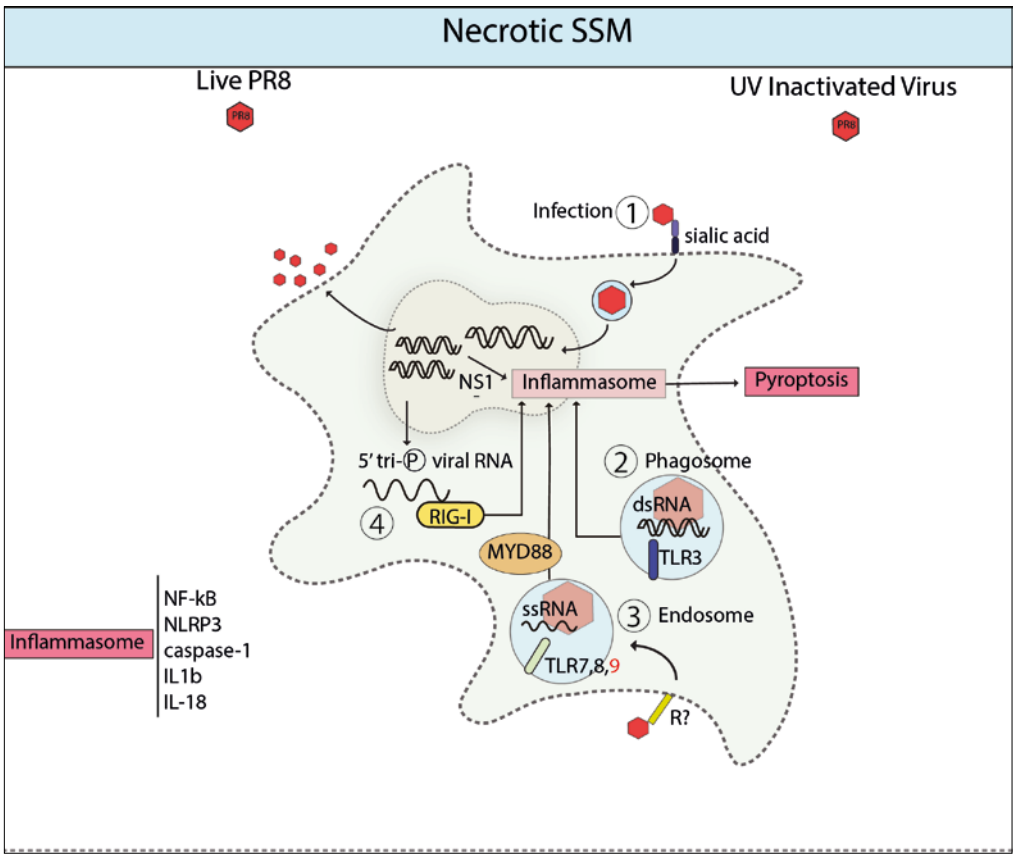


Figure 5
Graphic representation of the activation of the inflammasome pathway in lymph node macrophages following influenza administration.

Funding

European Union - Marie Curie CIG
IRINVAC: Immune Responses to Influenza Vaccine.
FP7-PEOPLE-2013-CIG / 2013-2017

Swiss National Science Foundation - Ambizione
IMPORED: Intravital Imaging of Polymicrobial Respiratory Diseases.
148183 / 2014-2016

SystemsX.ch
A massively parallel space-time connected approach based on implicit active contour methods to track leukocytes observed by multiphoton intravital and confocal microscopy / 2013-2016

Collaborations

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

Andrea Cerutti
The Mount Sinai Hospital, New York (US)

Adolfo Garcia-Sastre
The Mount Sinai Hospital, New York (US)

Cornelia Halin
ETH Zurich (CH)

Annelies Zinkernagel
University Hospital Zurich (CH)

Maria Jose Alonso
University of Santiago de Compostela (ES)

Amelia Nieto
Centro Nacional de Biotecnologia (ES)

Visiting Scientist

Jordi Sintes Castro
Barcelona Biomedical Research Park (ES)

Publications

Antibody-mediated immunity
Baumgarth, N., M. C. Carroll and S. González (2013).
Textbook of Influenza, 2nd Edition (eds R. G. Webster, A. S. Monto, T. J. Braciale and R. A. Lamb), John Wiley & Sons, Ltd, Oxford, UK.

Endocytosis and recycling of immune complexes by follicular dendritic cells enhances B cell antigen binding and activation
Heesters B. A., P. Chatterjee, Y. A. Kim, S. F. González, M. P. Kuligowski, T. Kirchhausen and M. C. Carroll.
Immunity. 2013; 38:1164-1175.

Cochlin Produced by follicular dendritic cells promotes anti-bacterial innate immunity.
Py B. F., S. F. González, K. Long, Y. Kim, J. Yao, H. Zhu, N. Degauque, R. Villet, Y.-L. Patrick, M.-S. Ki, M. Gadjeva, G. B. Pier, M. Carroll and J.Yuan.
Immunity. 2013; 38;1063-1072.

Lectures and seminars

10th International Conference on Innate Immunity
"Complement C3 is necessary to promote phagocytosis of influenza virus by the major phagocytic cells in the lungs"
Kos (GR) / 24.06.2013

Retreat MIM Program ETH Zurich
"A 2-photon intravital approach to image the transport of antigen in the lymph node"
Locarno (CH) / 13.07.2013

ETH Zurich
"Antigen trafficking in the lymph node"
Zurich (CH) / 19.11.2013

EADV meeting
"Role of neutrophils in vaccine immunity"
Bellinzona (CH) / 28.11.2013

Centro Nacional de Biotecnologia
"An intravital approach to study the immune response after vaccination"
Madrid (ES) / 19.09.2014

1st International meeting on imaging the immune system
"Imaging cell dynamics in the lymph node after vaccination"
Milan (IT) / 23.08.2014

EADV meeting
"Role of Macrophage-neutrophil interaction in the initiation of the immune response against influenza vaccine"
Bellinzona (CH) / 4.12.2014.

University of Santiago de Compostela
"In vivo imaging of antigen trafficking in the lymphatic compartment"
Santiago de Compostela (ES) / 22.12.2014

Organization of International Congresses

1st International meeting on imaging the immune system
San Raffaele Scientific Institute, Milan (IT) / 23-24.08.2014

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. He heads the T Cell Development lab at IRB. His research is focused on the purinergic regulation of various aspects of T cell physiology, including signal transduction, control of cell growth and regulation of host-microbiota mutualism in the gut.

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. Its prolonged stimulation or high concentration of ATP determine the opening of a pore permeable to molecules up to 900 Da and cell death. We aim at understanding the role of P2X7 in regulating T cell homeostasis and adaptive immunity in different physiological and pathological conditions. At the moment we are addressing the role of P2X7 in regulating T follicular helper (Tfh) cells and secretory IgA response in the intestine. Moreover, we are investigating the role of P2X7 in conditioning type 1 diabetes onset and progression.

Team

Group Leader: Fabio Grassi, MD, PhD > fabio.grassi@irb.usi.ch
Members: Caterina Elisa Faliti, PhD student - Lisa Perruzza, PhD student - Michele Proietti, MD, PhD - Tanja Rezzonico Jost, Staff scientist - Andrea Romagnani, PhD student - Elsa Rottoli, PhD student.

Regulation of mucosal immunity in the gut by purinergic P2X7 receptor

Michele Proietti, Tanja Rezzonico Jost, Andrea Romagnani, Lisa Perruzza, Caterina E. Faliti and Fabio Grassi

Host's physiology and diet influence the initial development of the 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. The molecular mechanisms, which regulate GALT function and result in gut-commensal homeostasis are poorly defined. T follicular helper (Tfh) cells in Peyer's patches (PPs) promote high-affinity IgA responses. We have shown that the ATP-gated ionotropic P2X7 receptor regulates Tfh cells abundance in PPs. Lack of P2X7 in Tfh cells enhances the germinal centre reaction, high affinity IgA secretion and binding to commensals. The ensuing depletion of mucosal bacteria results in reduced systemic translocation of microbial components, which affects B1 cells stimulation and serum IgM levels. Mice lacking P2X7 succumb to polymicrobial sepsis by otherwise sublethal caecal ligation and puncture (CLP). Depletion of Tfh cells or administration of purified IgM rescue P2X7 deficient mice from CLP, indicating that regulation of Tfh cells by P2X7 activity is important for mucosal colonization, which in turn results in IgM serum levels necessary to protect the host from bacteraemia (Figure 1).

*** *Proietti M. et al.***
Immunity.
2014, 41: 789-801.

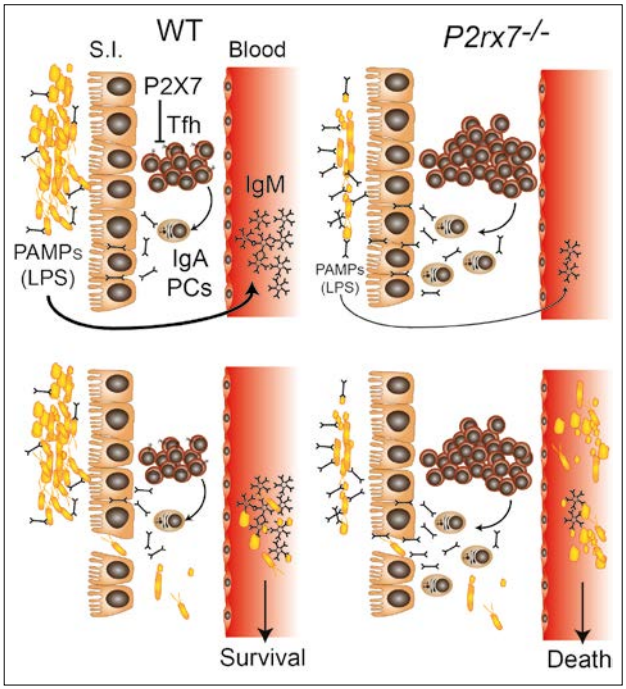


Figure 1
Regulation of T follicular helper cells in Peyer's patches by endoluminal ATP and P2X7 signaling.
Upper panels show inhibition of T cell dependent IgA production in the small intestine by endoluminal ATP via P2X7 to preserve commensalism. Lack of P2X7 in Tfh cells results in depletion of mucosal commensals and low serum IgM by reduced absorption of microbial products (PAMP, pathogen associated microbial pattern; LPS, lipopolysaccharide). Lower panels show that mucosal colonization in WT but not p2rx7$^{-/-}$ mice results in physiological serum concentrations of IgM that ensure protection from polymicrobial sepsis by sublethal bacteraemia.

Regulation of glucose metabolism by P2X7 activity in T follicular helper cells in the small intestine

Lisa Perruzza, Michele Proietti and Fabio Grassi

The interplay between the epithelial component gut associated immune system and microbiota ensures the physiological control of energy uptake, immune system activation and microbial commensalism. An altered responsiveness of any of these three components can lead to inflammatory conditions as well as metabolic syndrome-like phenotype. *P2rx7*$^{-/-}$ mice housed at IRB spf facility display features reminiscent of metabolic syndrome, including increased body weight, blood glucose and insulin levels, and fat accumulation, as measured by white adipose tissue (WAT) weight. This phenotype further deteriorated after 3 weeks of high-fat diet and improved after treatment with broad-spectrum antibiotics, thereby suggesting the possible implication of the microbiota in determining the metabolic phenotype. Analysis of the gut microbiota with genus specific probes revealed a 3-fold increase in the ratio between Firmicutes and Bacteroides. We defined differences in taxonomic composition of intestinal microflora in WT versus *p2rx7*$^{-/-}$ mice by pyrosequencing of bacterial 16S rRNA fragments. Short-chain fatty acids (SCFAs) are produced by microbiota in the colon and the distal small intestine from resistant starch, dietary fiber, and other low-digestible polysaccharides in a fermentation process. Acetate, propionate, and butyrate are the predominant SCFAs in the gut lumen in humans and rodents, and are present at high mM levels. To address whether alterations in short chain fatty acids abundance in the intestine of *p2rx7*$^{-/-}$ mice might play a role in the observed metabolic unbalance, we measured variations in relative abundance of butyrate, acetate and propionate in intestine contents from WT and *p2rx7*$^{-/-}$ mice. The Tfh cells were responsible cell autonomously for the metabolic alterations observed in *p2rx7*$^{-/-}$ mice since *cd3ε*$^{-/-}$ mice reconstituted with Tfh cells from *p2rx7*$^{-/-}$ mice showed body weight and blood glucose variation analogous to *p2rx7*$^{-/-}$ mice as well as increase in perigonadal WAT and impaired glucose tolerance test (GTT). Finally, fecal transplant experiments revealed the role played by altered microbiota in determining the P2X7-deficient phenotype. These results suggest that regulation of Tfh cells activity by P2X7 has a profound impact on microbiota composition and metabolic balance of the organism.

Purinergic P2X7 receptor in type 1 diabetes

Lisa Perruzza, Caterina E. Faliti 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.

***Vergani A. et al.** **
Diabetes. 2013, 62:1665-1675.

Modulation of effector/memory T cell responsiveness by P2X7 activity

Andrea Romagnani, Elsa Rottoli and Fabio Grassi

T effector/memory (TEM) cells express high levels of *p2rx7* transcript and P2X7 stimulation results in cell death. This outcome is prevented by TCR stimulation because of robust downregulation of p2rx7. TCR triggering leads to the activation of different protein kinases including upstream src-family kinases LCK and FYN. These activated proteins orchestrate the activation of different pathways that together ensure the proper activation and differentiation of T cells. The treatment of TEM cells with PP2 as a src-like kinase pharmacological inhibitor resulted in inhibition of CD3/CD28 mediated down modulation of *p2rx7* transcription indicating that LCK/FYN signaling is required for p2rx7 transcriptional regulation. Naïve T cells injected into lymphopenic hosts acquire phenotypic characteristics of memory cells and undergo extensive proliferation, referred to as homeostatic expansion. Deletion of *p2rx7* confers enhanced reconstitution potential to T cells. Moreover, *p2rx7*$^{-/-}$ cells were characterized by reduced secretion of pro inflammatory cytokines with respect to wild-type cells. In addition, lack of *p2rx7* confers increased proliferative and survival potentials to T cells in response to cognate Ag stimulation. Two important parameters related to cellular metabolism are extracellular acidification rate (ECAR) that is an indicator of lactic acid production by glycolysis, and oxygen consumption rate (OCR), a direct indicator of mitochondrial respiration. Both ECAR and OCR were increased in *p2rx7*$^{-/-}$ TEM cells, indicating that lack of P2X7 activity confers a bioenergetic advantage. Altogether, these results point to P2X7 as an important regulator of T cell responsiveness in both homeostatic conditions and upon cognate antigen encounter.

Purinergic antagonism in muscular dystrophy

Lisa Perruzza 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.

Control of intraepithelial T cell function by the kinase activity of Transient receptor potential melastatin-like 7 (Trpm7) ion channel

Andrea Romagnani and Fabio Grassi

The magnesium (Mg^{2+}) and calcium (Ca^{2+}) conducting transient receptor potential channel-enzyme TRPM7 has a vital role in cellular physiology. However, the regulation of the protein's channel or enzymatic function is poorly understood. Unlike conventional kinases, TRPM7 has no specific (AA-) motif but phosphorylates serines and threonines located within alpha-helices. It contains a Ser/Thr-rich autophosphorylation site, which is important for kinase activity itself. It phosphorylates the anti-inflammatory molecule annexin A1 that regulates effector functions of immune cells. Mg^{2+} and Mg^{2+}ATP provide a negative feedback loop by regulating the channel's activity. Several binding sites for Mg^{2+} and Mg^{2+}ATP within the kinase domain have been identified. We are using a homozygous kinase-dead mouse model, *TRPM7^{KI}*, with a single point mutation at one of these sites. Unlike mice lacking the entire kinase domain, homozygous *TRPM7^{KI}* mice are viable. They are normal in size, weight, fur color, and Mendelian inheritance ratio compared to wild-type (wt) animals. We excluded a role of the kinase activity in the regulation of biophysical features of the TRPM7 channel. Nevertheless, we were able to demonstrate that *TRPM7^{KI}* channels display a slightly decreased Mg^{2+}-sensitivity without causing differences in main electrolyte concentrations in *TRPM7^{KI}* vs wt mice. The morphology and lymphocyte cellularity of Peyer's patches (PPs) were the most prominent changes we found in *TRPM7^{KI}* mice. PPs from *TRPM7^{KI}* mice were hypotrophic and showed complete lack of germinal centers, resulting in reduced amount of secreted IgA in the small intestine. To study the role of TRPM7 in GALTs homeostasis *in vivo* we used an established experimental colitis model, namely dextran sodium sulphate (DSS) induced colitis. We observed a dramatic outcome of DSS-colitis in *TRPM7^{KI}* mice, which lost significantly more weight and showed more severe rectal bleeding than wt mice. All *TRPM7^{KI}* mice died within 10 days from the beginning of the experiment. Intraepithelial T cells (IEL) were particularly affected by lack of TRPM7 kinase activity and CD103 expression was strongly reduced. Interestingly, adoptive transfer of lymphopenic host with naïve T cells from *TRPM7^{KI}* mice did not recover CD103 expression or reconstitute the IEL pool in gut epithelium, suggesting that TRPM7 kinase activity plays a fundamental role in T cell colonization and patrolling of gut epithelium. Taken together, our results suggest that TRPM7 kinase activity regulates intraepithelial T cell function.

Mucosal immunity in the pathogenesis of Omenn Syndrome

Fabio Grassi

Omenn syndrome (OS) is a rare monogenic disorder associating immunodeficiency and autoimmune-like manifestations that can present in the form of an inflammatory bowel disease-like illness. Although the identification of the molecular bases of OS has allowed the precise diagnosis of the disease, the complete comprehension of the molecular and cellular mechanisms underlying OS autoimmunity is still puzzling. In this regard, one of the most intriguing and yet unsolved questions is whether and how an altered homeostasis of the intestinal system influences the pathogenesis of OS autoimmunity, thus ultimately affecting the clinical phenotype. In OS and more generally in immunodeficient patients, prolonged courses of antibiotic may affect the development of functional regulatory T cells and in parallel forge the microbiota plasticity resulting in dysbiosis. Compelling evidences demonstrate a link between this phenomenon, chronic immune inflammation and autoimmune disease onset/progression in genetically susceptible hosts. The development of the Rag2$^{R229Q/R229Q}$ mouse, a faithful model of the human disease, allows us to directly tackle this unexplored aspect in OS pathogenesis. We observed an abnormal leukocytes trafficking to the intestinal mucosa, associated to the inflammatory state of both small and large intestines. Increased cellularity of CD4 T cells and, intriguingly, of Foxp3+ T regulatory (Treg) cells in the Rag2$^{R229Q/R229Q}$ mice is a unique feature of the lamina propria compartment, sharply contrasting with the overall lymphoid depletion observed in the other peripheral lymphoid organs. Moreover, we found that mucosal IgA levels are markedly reduced in the mutant mice. We hypothesize that dysfunctions of the regulatory mechanisms maintaining the balance between active immunity and tolerance in the gut lead to defects in the gut-blood barrier that might render OS patients susceptible to inflammation sustained by endogenous commensal flora and promote both local and systemic autoimmunity.

Funding

Swiss National Science Foundation
Purinergic control of adaptive immunity by P2X7 receptor
310030_124745 / 2012-2015

ERA.Net RUS
TIROTAPS: TRPM7 in Regulation of T cell subsets and Purinergic Signaling
No. 184 / 2012-2014

Fondazione San Salvatore
Identification of novel targets in central nervous system infiltration in acute T lymphoblastic leukaemia
2012-2014

Collaborations

Andrea Alimonti
Institute of Oncology Research (IOR), Bellinzona (CH)

Claudio Bruno, Elisabetta Gazzerro
Istituto Giannina Gaslini, Genoa (IT)

Sandro Carrara, Giovanni De Micheli
Swiss Federal Institute of Technology, Lausanne (CH)

Lucienne Chatenoud
Université Paris Descartes, INSERM, Paris (FR)

Catherine Dehollain
Swiss Federal Institute of Technology, Lausanne (CH)

Ursula Grohmann, Francesca Fallarino
Department of Experimental Medicine, Università degli Studi di Perugia (IT)

Leif Groop, João Fadista
Lund University Diabetes Centre, CRC, Malmö University Hospital (SE)

Michela Matteoli,
Department of Medical Pharmacology, Università degli Studi di Milano (IT)

Kathy D. McCoy
UVCM, University of Bern (CH)

Graziano Pesole
Italian National Research Council (CNR), Università di Bari (IT)

Camillo Ricordi
Cell Transplant Center and Diabetes Research Institute, University of Miami, FL (US)

Emma Slack
Institute of Microbiology, ETH Zurich (CH)

Gabriella Tedeschi
Department of Animal Pathology, Hygiene and Veterinary Public Health, Università degli Studi di Milano (IT)

Elisabetta Traggiai
Novartis Institute for Research in Biomedicine, Basel (CH)

Claudia Verderio
Italian National Research Council (CNR), Institute of Neuroscience, Milan (IT)

Anna Villa
Italian National Research Council (CNR), Istituto Clinico Humanitas, Rozzano (IT)

Ursula von Mandach
Department of Obstetrics and Gynecology, University Hospital Zurich (CH)

Susanna Zierler
Walther-Straub-Institute for Pharmacology and Toxicology, LMU Munich (DE)

Publications

ATP-Gated Ionotropic P2X7 Receptor Controls Follicular T Helper Cell Numbers in Peyer's Patches to Promote Host-Microbiota Mutualism.
Proietti, M., V. Cornacchione, T. Rezzonico Jost, A. Romagnani, C. E. Faliti, L. Perruzza, R. Rigoni, E. Radaelli, F. Caprioli, S. Preziuso, B. Brannetti, M. Thelen, K. D. McCoy, E. Slack, E. Traggiai and F. Grassi
Immunity. 2014; 41:789-801.

LPS Nephropathy in Mice Is Ameliorated by IL-2 Independently of Regulatory T Cells Activity.
Bertelli, R., A. Di Donato, M. Cioni, F. Grassi, M. Ikehata, A. Bonanni, M. P. Rastaldi and G. M. Ghiggeri
PLoS One. 2014; 9:e111285.

Full fabrication and packaging of an implantable multi-panel device for monitoring of metabolites in small animals.
Baj-Rossi, C., E. G. Kilinc, S. S. Ghoreishizadeh, D. Casarino, T. R. Jost, C. Dehollain, F. Grassi, L. Pastorino, G. De Micheli and S. Carrara
IEEE Trans Biomed Circuits Syst. 2014; 8:636-647.

Enhancing chemotherapy efficacy in Pten-deficient prostate tumors by activating the senescence-associated antitumor immunity.
Toso, A., A. Revandkar, D. Di Mitri, I. Guccini, M. Proietti, M. Sarti, S. Pinton, J. Zhang, M. Kalathur, G. Civenni, D. Jarrossay, E. Montani, C. Marini, R. Garcia-Escudero, E. Scanziani, F. Grassi, P. P. Pandolfi, C. V. Catapano and A. Alimonti
Cell Rep. 2014; 9:75-89.

Rag Defects and Thymic Stroma: Lessons from Animal Models.
Marrella, V., P. L. Poliani, L. D. Notarangelo, F. Grassi and A. Villa
Front Immunol. 2014; 5:407.

Tumour-infiltrating Gr-1+ myeloid cells antagonize senescence in cancer.
Di Mitri, D., A. Toso, J. J. Chen, M. Sarti, S. Pinton, T. R. Jost, R. D'Antuono, E. Montani, R. Garcia-Escudero, I. Guccini, S. Da Silva-Alvarez, M. Collado, M. Eisenberger, Z. Zhang, C. Catapano, F. Grassi and A. Alimonti
Nature. 2014; 515:134-137.

Continuous monitoring of Naproxen by a cytochrome P450-based electrochemical sensor.
Baj-Rossi, C., T. Rezzonico Jost, A. Cavallini, F. Grassi, G. De Micheli and S. Carrara
Biosens Bioelectron. 2014; 53:283-287.

Dependence of immunoglobulin class switch recombination in B cells on vesicular release of ATP and CD73 ectonucleotidase activity.
Schena, F., S. Volpi, C. E. Faliti, F. Penco, S. Santi, M. Proietti, U. Schenk, G. Damonte, A. Salis, M. Bellotti, F. Fais, C. Tenca, M. Gattorno, H. Eibel, M. Rizzi, K. Warnatz, M. Idzko, C. K. Ayata, M. Rakhmanov, T. Galli, A. Martini, M. Canossa, F. Grassi and E. Traggiai
Cell Rep. 2013; 3:1824-1831.

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.

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; 62:1665-1675.

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.

Lectures and Seminars

2nd International Aegean Conference on Immuno-Metabolism: Molecular and Cellular Immunology of Metabolism
"Regulation of mucosal IgA response and host-microbiota mutualism in the small intestine by endoluminal ATP"
Rhodes, Greece / 15-20.09.2013

Novartis Institutes for Biomedical Research
"Pleiotropic role of ATP as signaling molecule in adaptive immunity via P2X receptor"
Basel, Switzerland / 10.04.2014

Istituto Nazionale Genetica Molecolare
"Pleiotropic role of ATP as signaling molecule in adaptive immunity via P2X receptor"
Milan (IT) / 24.07.2014

XXIV AINI Congress
"Astrocyte-T cell cross-talk"
Sorrento (IT) / 01-04.10.2014

Department of Pharmacology, University of Milan
"Stage specific regulation of T cell by purinergic P2X activity"
Milan (IT) / 06.02.2015

Antonio Lanzavecchia
Immune Regulation

Antonio Lanzavecchia

Antonio Lanzavecchia earned a degree in Medicine at the University of Pavia where he specialized in Pediatrics and in Infectious Diseases. From 1983 to 1999, he worked at the Basel Institute for Immunology and since 1999 is the Director of the Institute for Research in Biomedicine in Bellinzona. He taught immunology at the Universities of Genoa 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 and the Cloëtta prize, 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

We are interested 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 for prophylaxis and treatment of infectious diseases, but also as tools to identify vaccine candidates. Besides these translational studies we address fundamental issues such as 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

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

Members: Sonia Barbieri, PhD - Costanza Casiraghi, PhD - Davide Corti, PhD - Blanca Fernandez-Rodriguez, technician - Mathilde Foglierini, Staff Scientist - Alexander Fruehwirth, PhD student - Roger Geiger, PhD - Isabella Giacchetto-Sasselli, technician - Valentina Gilardi PhD student - David Jarrossay, PhD - Sandra Jovic, technician - Daniele Lilleri, MD - Jessica Marcandalli, Technician - Esther Katelaars, master student - Andrea Minola, technician - Leontios Pappas, PhD student - Laurent Perez, PhD - Debora Pinna, PhD - Dora Pinto, PhD - Sara Ravasio, PhD student - Luca Piccoli, PhD - Kathrin Pieper, PhD - Chiara Silacci Fregni, technician - Joshua Hoong Yu Tan, PhD student - Tobias Wolf, PhD student.


high
important
cross-reactive
generated
maturation
antigenic
used
viral
chain
somatic
immunoglobulin
viruses
higher
antibodies
study
HA
GC
infection
reported
residues
sequences
diversity
whereby
stem
Influenza
found
following
diversification
neutralizing
target
able
although
levels
may
recently
plasma
surface
virus
cells
affinity
group
demonstrated
human
memory
selection
isolated
also
VHI-69
HCDR3
substitutions
SHM
mutations
antibody
specific
mutation
cell
broadly
binding
antigen
repertoire
different
HIV-1
responses
process
response

Antibody-driven design of a human cytomegalovirus gHgLpUL128L subunit vaccine that selectively elicits potent neutralizing antibodies

Anna Kabanova, Laurent Perez, Daniele Lilleri, Jessica Marcandalli, Gloria Agatic, Simone Becattini, Davide Corti and Antonio Lanzavecchia

Kabanova A. et al. *
PNAS. 2014, 111: 17965–17970

The use of neutralizing antibodies to identify the most effective antigen has been proposed as a strategy to design vaccines capable of eliciting protective B-cell immunity. In this study, we analyzed the human antibody response to cytomegalovirus (human cytomegalovirus, HCMV) infection and found that antibodies to glycoprotein gB, a surface glycoprotein that has been developed as a HCMV vaccine, were primarily non neutralizing. In contrast, most of the antibodies to the complex formed by gH, gL, protein pUL128, pUL130, and pUL131 (the gHgLpUL128L pentamer) neutralized HCMV infection with high potency. Based on this analysis, we developed a single polycistronic vector encoding the five pentamer genes separated by "self-cleaving" 2A peptides to generate a stably transfected CHO cell line constitutively secreting high levels of recombinant pentamer that displayed the functional antigenic sites targeted by human neutralizing antibodies. Immunization of mice with the pentamer formulated with different adjuvants elicited HCMV neutralizing antibody titers that persisted to high levels over time and that were a hundred- to thousand-fold higher than those found in individuals that recovered from primary HCMV infection. Sera from mice immunized with the pentamer vaccine neutralized infection of both epithelial cells and fibroblasts and prevented cell-to-cell spread and viral dissemination from endothelial cells to leukocytes. Neutralizing monoclonal antibodies from immunized mice showed the same potency as human antibodies and targeted the same as well as additional sites on the pentamer. These results illustrate with a relevant example a general and practical approach of analytic vaccinology for the development of subunit vaccines against complex pathogens.

This work was done in collaboration with Silvia Preite and Federica Sallusto, IRB, and Elena Percivalle and Giuseppe Gerna, Fondazione IRCCS Policlinico San Matteo, Pavia (IT).

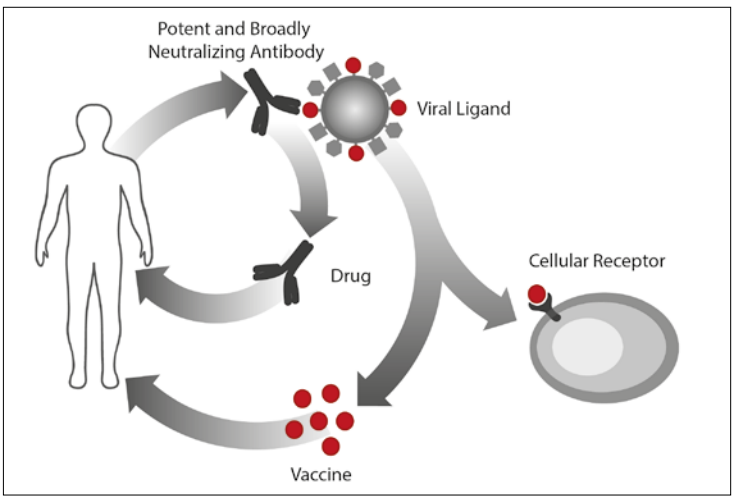


Figure 2
The use of neutralizing antibodies for serotherapy, vaccine design and viral receptor discovery

Cross-neutralization of four paramyxoviruses by a human monoclonal antibody

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

Broadly neutralizing antibodies reactive against most and even all variants of the same viral species have been described for influenza and HIV-1. However, whether a neutralizing antibody could have the breadth of range to target different viral species was unknown. 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, and play a role in asthma exacerbations. Although antisera generated against either HRSV or HMPV are not cross neutralizing, we speculated that, because of the repeated exposure to these viruses, cross-neutralizing antibodies might be selected in some individuals. Here we describe a human monoclonal antibody (MPE8) that potently cross-neutralizes HRSV and HMPV as well as two animal paramyxoviruses: bovine RSV (BRSV) and pneumonia virus of mice (PVM). In its germline configuration, MPE8 is HRSV-specific and its breadth is achieved by somatic mutations in the light chain variable region. MPE8 did not result in the selection of viral escape mutants that evaded antibody targeting and showed potent prophylactic efficacy in animal models of HRSV and HMPV infection, as well as prophylactic and therapeutic efficacy in the more relevant model of lethal PVM infection. The core epitope of MPE8 was mapped on two highly conserved anti-parallel β-strands on the pre-fusion viral F protein, which are rearranged in the post-fusion F protein conformation. Twenty-six out of the thirty HRSV-specific neutralizing antibodies isolated were also found to be specific for the pre-fusion F protein. Taken together, these results indicate that MPE8 might be used for the prophylaxis and therapy of severe HRSV and HMPV infections and identify the pre-fusion F protein as a candidate HRSV vaccine.

* *Corti D. et al.*
Nature. 2013, 501: 439-443 2013

This work was 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).

Rapid development of broadly influenza neutralizing antibodies through redundant mutations

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

Pappas L. et al. *
Nature. 2014, 516:418-422

The neutralizing antibody response to influenza virus is dominated by antibodies that bind to the globular head of haemagglutinin, which undergoes a continuous antigenic drift, necessitating the re-formulation of influenza vaccines on an annual basis. Recently, several laboratories have described a new class of rare influenza-neutralizing antibodies that target a conserved site in the haemagglutinin stem. Most of these antibodies use the heavy-chain variable region VH1-69 gene, and structural data demonstrate that they bind to the haemagglutinin stem through conserved heavy-chain complementarity determining region (HCDR) residues. However, the VH1-69 antibodies are highly mutated and are produced by some but not all individuals, suggesting that several somatic mutations may be required for their development. To address this, here we characterize 197 anti-stem antibodies from a single donor, reconstruct the developmental pathways of several VH1-69 clones and identify two key elements that are required for the initial development of most VH1-69 antibodies: a polymorphic germline-encoded phenylalanine at position 54 and a conserved tyrosine at position 98 in HCDR3. Strikingly, in most cases a single proline to alanine mutation at position 52a in HCDR2 is sufficient to confer high affinity binding to the selecting H1 antigen, consistent with rapid affinity maturation. Surprisingly, additional favourable mutations continue to accumulate, increasing the breadth of reactivity and making both the initial mutations and phenylalanine at position 54 functionally redundant. These results define VH1-69 allele polymorphism, rearrangement of the VDJ gene segments and single somatic mutations as the three requirements for generating broadly neutralizing VH1-69 antibodies and reveal an unexpected redundancy in the affinity maturation process.

This work was done in collaboration with Filippo Turrini, Gabriele Pellicciotta and Elisa Vicenzi, HSR Milano (IT), and Nicole Kalleward and Qing Zhu MedImmune, Gaithensburg (US).
An example of a family of antibodies specific for influenza hemagglutinin is shown in figure 3.

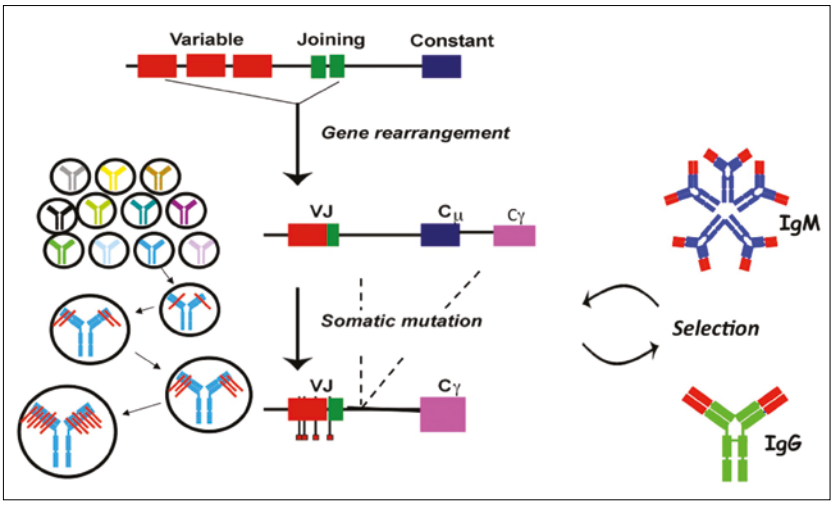


Figure 3
Generation of antibody diversity (Courtesy of Michael Neuberger)

Neutralization and clearance of GM-CSF by autoantibodies in pulmonary alveolar proteinosis

Luca Piccoli, Chiara Silacci Fregni, Blanca Fernandez-Rodriguez, Andrea Minola, Davide Corti and Antonio Lanzavecchia

Pulmonary alveolar proteinosis (PAP) is a severe autoimmune disease caused by autoantibodies that neutralize GM-CSF resulting in impaired function of alveolar macrophages. In this study, we characterize 21 GM-CSF autoantibodies from PAP patients and find that somatic mutations critically determine their specificity for the self-antigen. Individual antibodies only partially neutralize GM-CSF activity using an in vitro bioassay, depending on the experimental conditions, while, when injected in mice together with human GM-CSF, they lead to the accumulation of a large pool of circulating GM-CSF that remains partially bioavailable. In contrast, a combination of three non-cross-competing antibodies completely neutralizes GM-CSF activity in vitro by sequestering the cytokine in high-molecular-weight complexes, and in vivo promotes the rapid degradation of GM-CSF-containing immune complexes in an Fc-dependent manner. Taken together, these findings provide a plausible explanation for the severe phenotype of PAP patients and for the safety of treatments based on single anti-GM-CSF monoclonal antibodies. Based on the above observations, we designed bi-specific and tri-specific antibodies and tested them for their capacity to neutralize the biological activity of GM-CSF. We identified several constructs that neutralized GM-CSF completely and stoichiometrically being 100 fold more efficient than therapeutic antibodies currently in clinical trials. These finding provide a strong rationale to use bi- or tri-specific antibodies whenever complete neutralization of bioactive molecules must be achieved.

*** *Piccoli L. et al***
Nat. Commun. 2015, 6:7375.

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

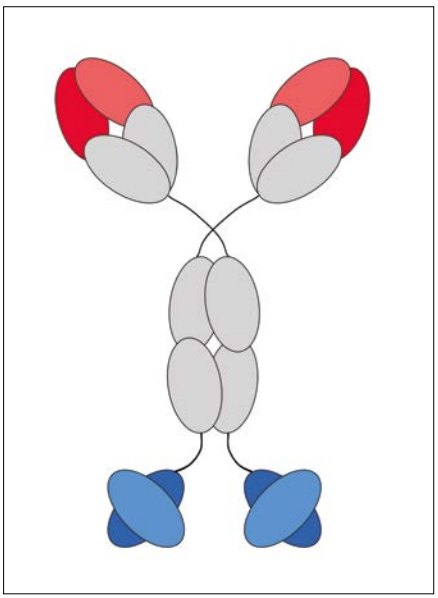


Figure 4
Engineered bispecific antibodies that potently neutralize GM-CSF

Dissecting the factors that limit the antibody response to the stem of influenza HA

Kathrin Pieper and Antonio Lanzavecchia

We previously identified three main structural elements that underpin the broadly neutralizing antibody response to the stem of influenza HA, namely: i) a frequent VH1-69 allele, ii) a HCDR3 of 13 amino acids with a Y at position 98 and iii) a single P52aA mutation. These elements are consistent with a high frequency of specific precursors in the naïve B cell repertoire and rapid affinity maturation and therefore indicate that the antibody response to the stem is not limited by B cell intrinsic factors. Since the antibody response to the HA stem develops slowly and is subdominant, we are interested to determine which might be the extrinsic factors that limit this protective and highly desirable response. In particular we consider the possibility that the anti-stem response may be limited by the low abundance or instability of the relevant stem epitopes, which are exclusively present in the pre-fusion HA. To investigate the relative immunogenicity of pre- and post-fusion HA conformation we are immortalizing memory B cells from donors after influenza infection or vaccination and screen the antibodies for their capacity to stain virus-infected cells (H1N1, Ca09) as such, or after exposure to pH 5.0 to trigger the post-fusion conformation. We want to obtain a complete atlas of the antibody response to pre- and post-fusion conformations of HA. These antibodies will be useful to develop a serological assay to measure the different specificities present in immune sera and to determine the conformation of the antigens in the vaccine. As a follow up to this study we are considering the possibility of developing as a vaccine a stabilized pre-fusion HA. To produce such stabilized protein, we will combine several approaches, such as the introduction at the C-terminus of a trimerization domain, the introduction of inter- and intra-monomeric disulphide bonds, cavity filling by hydrophobic substitutions and random mutagenesis. The characterization of the mutant molecules will be facilitated by the use of conformation-sensitive monoclonal antibodies to probe construct stability under physical stress conditions.

This work is done in collaboration with Antonino Cassotta and Federica Sallusto, IRB.

Broadly neutralizing antibodies to human Parainfluenza viruses

Valentina Gilardi, Davide Corti and Antonio Lanzavecchia

Human parainfluenza viruses (hPIV1-4) represent the second main cause of hospitalization in children under 5 years of age suffering from a respiratory illness, second only to RSV and MPV. To study the basis of the protective antibody response and to identify broadly neutralizing antibodies, we immortalized memory B cells from selected donors and isolated neutralizing antibodies specific for PIV3, the virus that causes the most severe pathology. Most of these antibodies bind to the pre- but not post-fusion conformation of the F protein. Interestingly, we found that several neutralizing antibodies bound to the HN protein and were also broadly cross-reactive. This result was unexpected in view of the very low level of amino acid identity (only 22%) among the HN molecules. We are currently determining the breadth of neutralization on different human and animal paramyxoviruses and are mapping the epitopes recognized. These antibodies will be instrumental to design vaccines based on stabilized trimeric pre-fusion F proteins or on the tetrameric HN protein.

This work is done in collaboration with the Kwong Laboratory, NIAID, Bethesda (US).

Neutralization hierarchy, V gene usage and affinity maturation in the antibody response to the globular head of SOIV HA

Alexander Frühwirth, Esther Ketelaars, Davide Corti and Antonio Lanzavecchia

Having determined the factors that govern the neutralizing antibody response to the stem of influenza HA, it is important to find whether the same rules apply to the response against the head, which represents the most variable and immunodominant region. We are therefore analyzing, in different donors, the spectrum of antibodies produced in response to the 2009 SOIV HA. The antibodies are tested for neutralizing and hemagglutination inhibition activity and the epitopes recognized are mapped using cross-competition and isolation of virus escape mutants. We also reconstruct the genealogy tree of several clones and study the relationship between epitope specificity and the capacity of the antibody to recruit effector mechanisms such as complement dependent or antibody dependent cellular cytotoxicity (CDC or ADCC). From this analysis several main themes emerge. First, the antibody response is directed primarily to the Sa/Sb site that partially overlaps with the sialic acid binding site of the HA. Interestingly, this response comprises both neutralizing and non-neutralizing antibodies that have distinctive VH/VL gene usage and bind to the Sa/Sb site with different orientations. Second, in most cases high affinity binding was reached with one or just a few mutations, while in others cases somatic mutations were redundant since the unmutated ancestor had already high affinity binding. Third, using a large panel of antibodies that span the whole surface of the HA, we determined that the capacity to induce ADCC or CDC, to inhibition fusion or hemagglutination was dependent on the topology of epitope recognized. In conclusion, the preferential V-gene usage and the rapid affinity maturation observed for anti-stem antibodies apply also to the antibody response to different sites and is therefore a general property of the immune response to influenza HA.

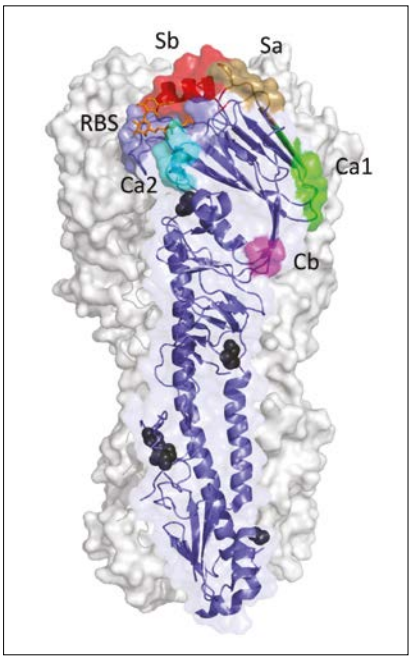


Figure 5
Antigenic sites of influenza hemagglutinin

Stabilized pre-fusion proteins as candidate vaccines

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

There is increasing evidence that the neutralizing antibody response is primarily directed against the pre-fusion conformation of viral glycoproteins and that, conversely, the antibody response to the post-fusion conformation gives only a minor contribution to total neutralizing activity and may even cause unwanted effects. These findings explain the failure of certain vaccines, such as the herpes and HCMV gB-based vaccines, which were based on post-fusion proteins. Therefore a rational approach to vaccine design is to produce stabilized pre-fusion proteins. To achieve this goal we collaborate with Peter Kwong (NIAID, Bethesda) to produce and test stabilized pre-fusion F proteins of paramyxoviruses (RSV, MPV, BRSV, PIV3). In addition several studies demonstrate that the immunogenicity of recombinant proteins can be increased by multimerization, for instance by display on virus-like particles. Therefore, in collaboration with David Baker (University of Washington, Seattle), we are developing constructs to display stabilized viral glycoproteins on protein nanostructures made by co-assembling multi-component proteins. Once expressed at sufficient levels, the nanoparticles will be tested for their capacity to induce virus neutralizing antibodies.

This work is done in collaboration with the Kwong Laboratory at NIAID, Bethesda (US) and the Baker laboratory, University of Washington, Seattle (US).

Protecting and pathogenic antibody responses to Dengue virus

Martina Beltramello 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.

* ***Williams K.L. et al***
PLoS Pathog. 2013, 9:e1003157

* ***Simonelli L. et al.***
PLoS ONE. 2013, 8:e55561.

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).

A potent broad-spectrum human monoclonal antibody cocktail for post-exposure prophylaxis and therapy of rabies

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

In humans, rabies prevention is achieved by vaccination or by post-exposure prophylaxis, wich consists in the administration of neutralizing antibodies together with an accelerated vaccination protocol. Currently, rabies-specific antibodies are of equine or human origin, the latter being considered safer but having high costs and limited availability. Therefore, there is a need to find potent and safe products. Memory B cells from a selected vaccinated donor were immortalized and monoclonal antibodies were screened for their affinity to the RABV G protein and tested for the recognition of G protein antigenic sites. Two antibodies RVC20 (antigenic site I) and RVC58 (antigenic site III) were selected for their breadth of reactivity and used for further analyses, along with currently available HRIG and three previously developed mAbs (CR57, CR4098 and 17C7) as a reference. RVC20 and RVC58 were able to neutralise all the 26 RABV isolates and 22 non-RABV lyssaviruses, with higher potency and breath compared to the reference tested. Furthermore, the RVC58 and RVC20 cocktail protected Syrian hamsters from a lethal RABV infection when used in substitution to HRIG, and did not affect the endogenous hamster post-vaccination antibody response to the RABV G protein. These results demonstrate that the human mAb cocktail of RVC20 and RVC58 are an efficacious alternative to RIG in human rabies PEP. Finally, in vivo experiments in Syrian Hamsters indicate that high doses of the antibody cocktail can cure animals that already had rabies virus in the CNS.

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

This work was done in collaboration with Paola De Benedictis, Roberta Aiello and Elena Rota Nodari, 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).

Structures of H5 influenza haemagglutinin in complex with a potent and broadly neutralizing human monoclonal antibody

Davide Corti, Debora Pinna, Mathilde Foglierini and Antonio Lanzavecchia

H5N1 avian influenza viruses remain a threat to public health mainly because they can cause severe infections of humans. They are widespread in birds, and they vary in antigenicity forming three major clades and numerous antigenic variants. The most important features of the human monoclonal antibody FLD194 studied here are its broad specificity for all major clades of H5 influenza HAs, its high affinity, and its ability to block virus infection, in vitro and in vivo. As a consequence it is the sort of antibody that would be suitable for anti-H5 therapy and as a component of stockpiles for Health authorities to use if an appropriate vaccine was not available. Our mutation and structural analyses indicate that the antibody recognizes a relatively conserved site near the membrane distal tip of HA, near to, but distinct from, the receptor binding site. They also suggest that the mechanism of infectivity neutralization involves prevention of receptor recognition as a result of steric hindrance by the Fc part of the antibody. Structural analyses by EM indicate that 3 Fab fragments are bound to each HA trimer. The structure revealed by X-ray crystallography is of a HA monomer bound by one Fab. The monomer has some similarities to HA in the fusion pH conformation and its formation, which results from the presence of isopropanol in the crystallization solvent, contributes to considerations of the process of change in conformation required for membrane fusion.

This work was done in collaboration with Steve Gamblin, Xiaoli Xiong Patrick J. Collins and John Skehel, The Francis Crick Institute, Mill Hill Laboratory, London (UK), Nigel J.Temperton, University of Kent (UK) and Amorsolo Suguitan and Kanta Subbarao, NIAID Bethesda (US).

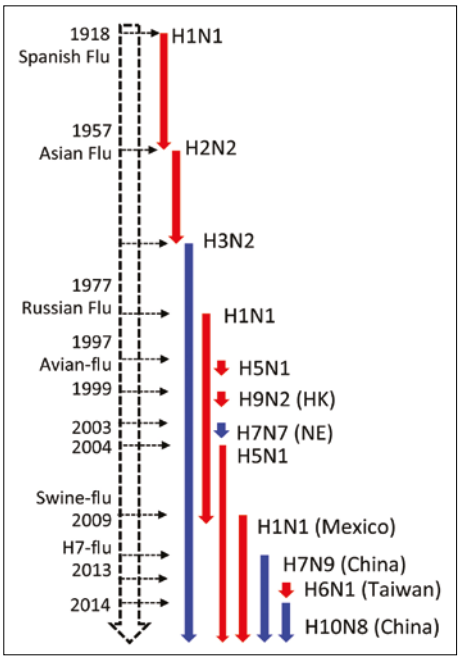


Figure 6
Pandemics, epidemics and outbreaks of influenza A in the last century

Immunogenetic mechanisms driving norovirus antigenic variation

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

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***
J Virology. 2014, 88: 8826–8842

* ***Debbink, K. et al***
J J Virology. 2014, 88:7244–7255

* ***Debbink, K. et al***
J Infec Dis. 2013, 208:1877–1887

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

A functional B cell receptor on human IgA and IgM plasma

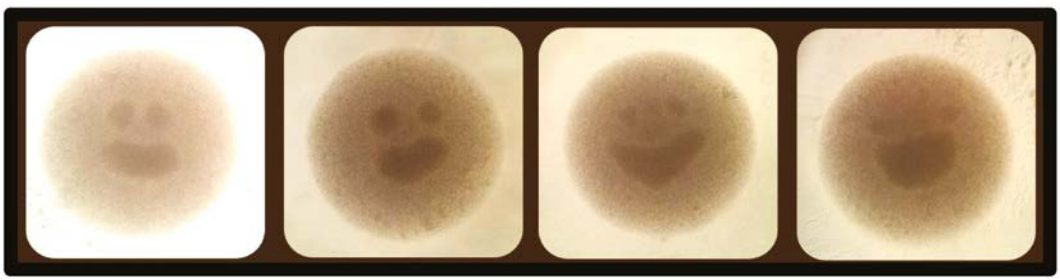
Dora Pinto, Antonio Lanzavecchia and David Jarrossay

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 Ca^{2+} 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 et al.***
Blood. 2013, 121:4110-4114

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

Figure 7
"Smiling" shapes detected in cell cultured T and B cells.



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. *
PloS One. 2013, 8:e59863.

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

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

Identification of recently activated B cells

Alexander Fruehwirth, Davide Corti and Antonio Lanzavecchia

Methods to separate recently activated B cells from the bulk of memory B cells would be useful to investigate the ongoing activity of the immune system in the steady state and in the course of the response to pathogens and would help to identify the factors that elicit and maintain the production of autoantibodies. To identify markers of recently activated B cells we used polychromatic flow cytometry and an unbiased analysis program to identify B cell subsets that increase following vaccination or infection. This approach was combined with the immortalization and analysis of the specificity of the sorted cells and the isolation of monoclonal antibodies. 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 antigen-specific memory cells. Using this method we will analyze the repertoire of recently activated B cells and total memory B cells in healthy individuals and in patients with chronic infections and autoimmune disorders.

The role of somatic mutations in the generation of autoantibodies

Luca Piccoli and Antonio Lanzavecchia

Somatic mutations of immunoglobulin V genes offer an efficient mechanism for affinity maturation and diversification leading to broadly neutralizing antibodies. However, they also carry the risk of generating autoantibodies. We suggest that autoreactive B cells that are fortuitously generated by somatic mutations and fail to be tolerized in germinal centers may enter the memory pool and be stimulated by self antigens found in peripheral tissues. Several factors may contribute to the expansion and differentiation of these autoreactive B cells, such as the release of self-antigens following necrotic or infectious events, genetic polymorphisms that facilitate the activation of memory B cells and the availability of cognate or non cognate T cell help. This model is supported by our finding that the unmutated common ancestor (UCA) of autoantibodies to desmoglein-3, GM-CSF and citrullinated vimentin do not bind to the self-antigens. This finding suggests that the autoantibodies were generated by somatic mutations in the course of the response to an unknown antigen. To identify the specificity of the UCA and thus reconstruct the genealogy tree of an autoantibody we will isolate a large panel of autoantibodies from patients with post-infectious autoimmune diseases such as Guillain-Barré syndrome (that can be preceded by infections with Campylobacter jejuni or influenza virus), or type II mixed cryoglobulinemia (that is caused by HCV infection and characterized by autoantibodies to IgG of the VH1-69 family). We will then assess whether the UCA of the autoantibodies can bind the infectious agent that has triggered the disease and, by reconstructing the genealogy tree, we will identify the mutations that had generated the autoantibody.

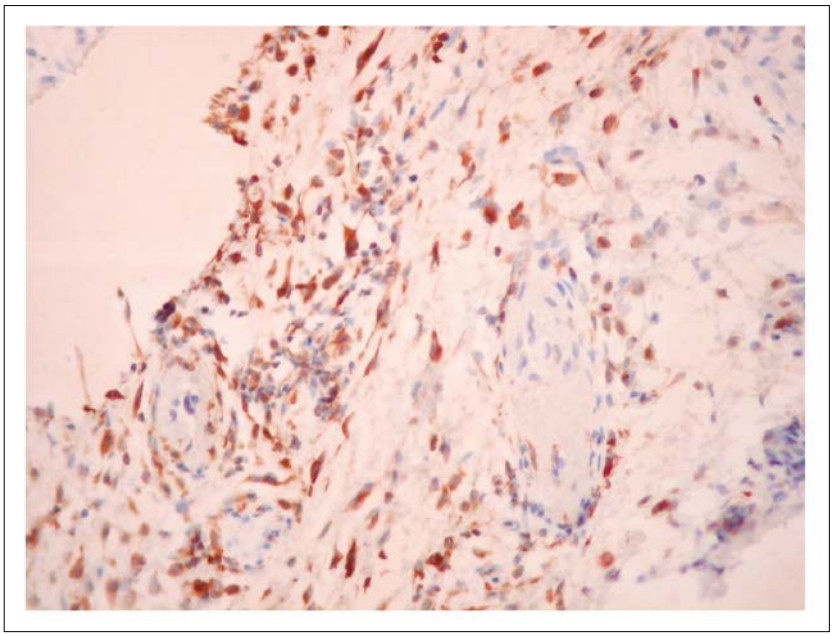


Figure 8
Inflamed synovial tissue from an RA patient stained by a monoclonal antibody to citrulinated vimentin

Investigation of Cross-reactive Antibodies Against Plasmodium falciparum-infected Erythrocytes

Joshua Tan, Kathrin Pieper, Luca Piccoli and Antonio Lanzavecchia

Antibodies against antigens exposed on *Plasmodium falciparum*-infected erythrocytes have been linked to protection against malaria. While the prevailing viewpoint is that such antibodies are predominantly variant-specific, a number of studies have reported that some of these antibodies can be partially cross-reactive. This observation led us to investigate the possibility that broadly cross-reactive antibodies exist in adults who live in regions with high malaria transmission. To achieve this aim, we utilized the mixed agglutination assay to screen plasma from a large Kenyan adult cohort against different parasite strains. A few adults possessing cross-reactive plasma were identified. IgG^+ and IgM^+ memory B cells from these donors were immortalized and screened for production of monoclonal antibodies of interest. We have identified several antibodies that show considerable breadth since they cross-react with erythrocytes infected with different field isolates. These antibodies have unusual properties that are actively investigated. We are also using these antibodies to identify the conserved antigens recognized, which will be potential novel candidates for malaria vaccine development.

This work is done in collaboration with Peter Bull, Nuffield Department of Medicine, Oxford University, Oxford (UK) and KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya (KE).

The neonatal Fc receptor enhances HIV-1 transcytosis across epithelial cells

Davide Corti and Antonio Lanzavecchia

Gupta E. et al *
PLoS Pathogens. 2013, 9e1003776

The mechanisms by which HIV-1 crosses mucosal surfaces to establish infection are unknown. Acidic genital secretions of HIV-1-infected women contain HIV-1 likely coated by antibody. We found that the combination of acidic pH and Env-specific IgG, including that from cervicovaginal and seminal fluids of HIV-1-infected individuals, augmented transcytosis across epithelial cells as much as 20-fold compared with Env-specific IgG at neutral pH or non-specific IgG at either pH. Enhanced transcytosis was observed with clinical HIV-1 isolates, including transmitted/founder strains, and was eliminated in neonatal Fc receptor (FcRn)-knockdown epithelial cells. Non-neutralizing antibodies allowed similar or less transcytosis than neutralizing antibodies. However, the ratio of total:infectious virus was higher for neutralizing antibodies, indicating that they allowed transcytosis while blocking infectivity of transcytosed virus. Immunocytochemistry revealed abundant FcRn expression in columnar epithelia lining the human endocervix and penile urethra. We conclude that acidity and Env-specific IgG enhance transcytosis of virus across epithelial cells via FcRn and could facilitate translocation of virus to susceptible target cells following sexual exposure.

This work is done in collaboration with Ruth Ruprecht, Texas Biomedical Research Institute, San Antonio (US), Dennis Burton, Ragon Institute, Boston (US), Jiri Mestecky, University of Alabama at Birmingham (US), Deborah Anderson, Boston University School of Medicine (US) and Donald Forthal, University of California, Irvine (US).

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 started in collaboration with Vito Pistoia, Istituto Gaslini, Genova (IT) and continues in collaboration with Elisabetta Cameroni and Davide Corti, Humabs BioMed, Bellinzona (CH).

Influence of L-arginine on metabolic networks and the lifespan of activated T cells

Roger Geiger, Tobias Wolf and Antonio Lanzavecchia

T cells play a crucial role in the defense against pathogens and tumors. Recently, metabolism has been linked to T cell fate and function but our knowledge regarding the dynamics of metabolic networks during the T cell response is limited. Here we used high-resolution mass spectrometry to follow 429 metabolites and 7,816 proteins after activation of primary human T cells. While numerous pathways were up-regulated, the only metabolic branch that decreased was L-arginine and four of its downstream metabolites. Interestingly, addition of exogenous L-arginine changed metabolism globally, including increased serine biosynthesis and a shift from glycolysis towards oxidative phosphorylation. It also promoted sirtuin-1 expression, endowed cells with a higher survival capacity and adoptively transferred tumor-specific mouse T cells exhibited improved anti-tumor activity. These profound phenotypic changes were likely induced by a complex ensemble of factors as L-arginine induced structural changes in 20 proteins. Our results provide a comprehensive resource for protein copy numbers and metabolite abundances in primary T cells and reveal that the common amino acid L-arginine is a key metabolic regulator.

This work is done in collaboration with Camilla Basso and Federica Sallusto, IRB, Bellinzona (CH); Jan Rieckmann, Felix Meissner and Matthias Mann, Max Planck Institute of Biochemistry, Martinsried (DE); Yuehan Feng and Paola Picotti Institute of Biochemistry, ETH Zürich (CH); Maria Kogadeeva and Nicola Zamboni, Institute of Molecular Systems Biology, ETH Zürich (CH).

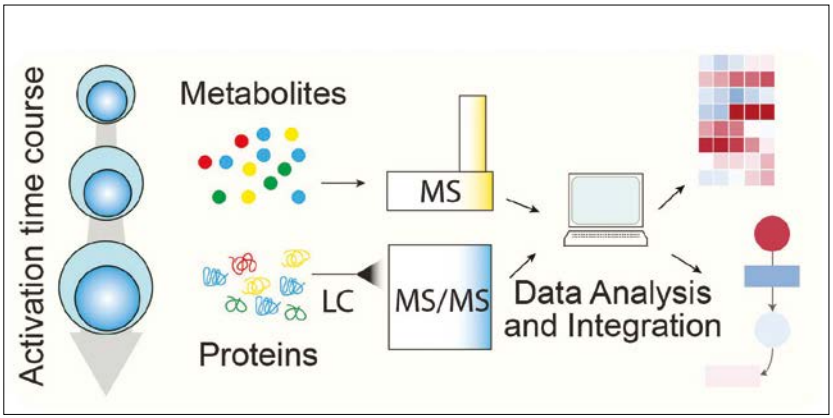


Figure 9
Proteomics and metabolomics analysis of T cell activation

Revitalizing exhausted anti-Tumor T cells for Cancer Immunotherapy

Tobias Wolf, Roger Geiger and Antonio Lanzavecchia

Malignant tumors harbor genetic mutations that can lead to the expression of neo-antigens, which are potentially recognized by the immune system. However, tumors often suppress immune attacks, for example through induction of inhibitory receptors on T cells, such as PD-1 or CTLA-4. Remarkably, blockade of these "immune-checkpoint" receptors for the treatment of cancer has yielded significant clinical benefits. Yet, T cell suppression in the tumor microenvironment can go beyond signaling through inhibitory receptors resulting in exhausted T cells that are intrinsically different and no longer respond to activation stimuli. Finding ways to revitalize exhausted tumor-infiltrated T cells (TILs) would greatly complement existing anti-cancer immune therapies. In this project we focus on hepatocellular carcinoma (HCC), which is the third leading cause of cancer-associated death worldwide. Standard therapies for the treatment of HCC are liver transplantation or resection, however HCC is rarely cured and recurs frequently. Several lines of evidence indicate that there is an existing immune response against HCC, offering the possibility to develop immune therapies by boosting this response. The goal is to develop methods to reactivate exhausted TILs isolated from primary tumors from HCC patients by identifying possible targets through a mass spectrometry approach. We will analyze proteomes of TILs and, as a reference, proteomes of circulating T cells from the patient's blood. A comparison of these two T cell types offers insights into the molecular underpinnings of T cell exhaustion and may reveal regulatory proteins that can be pharmacologically targeted to revitalize TILs. The second goal is to sequence the T cell receptor (TCR) repertoire of HCC-infiltrating T cells and express these sequences in functional T cells isolated from the patient's blood. T cells containing a grafted TCR that show specificity for tumor-antigens may be considered for therapeutic applications. While pursuing these goals of improving anti-cancer immune therapies, this project aims to additionally contribute to a better understanding of T cell exhaustion in the tumor microenvironment, and to provide knowledge about the TCR repertoire against HCC.

This work is done in collaboration with Federica Sallusto, IRB, Bellinzona; Elisabetta Loggi Department of Medical and Surgical Sciences, University of Bologna, Bologna (IT) ; Jan Rieckmann, Felix Meissner and Matthias Mann, Max Planck Institute of Biochemistry, Martinsried (DE).

Funding

CARIPLO Foundation
Development of a human cytomegalovirus vaccine based on glycoprotein complex including the UL128-131A gene products / 2010-2013

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

European Union
Innovative Medicines Initiative (IMI) Joint Undertaking (JU)
ABIRISK: Anti-Biopharmaceutical Immunization: Prediction and analysis of clinical relevance to minimize the risk
IMI-2010- Call3- 115303 / 2012-2017

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

National Institute of Health (US)
Vaccine-induced immunity in the young and aged
U19 AI057266-06 / 2009-2014

National Institute of Health (US)
Susceptibility and protective immunity to Noroviruses
2R01AI056351-06A1-#5-30027 / 2009-2014

Fondazione Adiuvare
L'origine e la patogenicità degli autoanticorpi nel Pemfigo, nella proteinosi Alveolare e in altre patologie organo-specifiche / 2013-2015

SystemsX.ch
Metabolic Regulations of Human T cell Activation and Differentiation / 2012-2015

Swiss Vaccine Research Institute
A target-agnostic approach to identify candidate vaccine antigens expressed at different stages of the P. falciparum life cycle using monoclonal antibodies from African volunteers experimentally infected with live sporozoites / 2014-2016

Swiss Vaccine Research Institute
Antibody discovery and engineering

Collaborations

Massimiliano Pagani
Fondazione INGM, National Institute of Molecular Genetics, Milan (IT)

Ralf Baric
University of North Carolina, Chapel Hill (US)

Herve Bourhy
Institut Pasteur, Paris (FR)

Paola De Benedictis
Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (IT)

Eva Harris
University of California, Berkeley, CA (US)

Fausto Baldanti
Fondazione IRCCS Policlinico San Matteo, Pavia (IT)

David Baker
University of Washington, Seattle (USA)

Giuseppe Gerna
Fondazione IRCCS Policlinico San Matteo, Pavia (IT)

Maurizio Luisetti
Fondazione IRCCS Policlinico San Matteo, Pavia (IT)

Pietro Speziale
University of Pavia, Pavia (IT)

Vito Pistoia
Istituto Gaslini, Genova (IT)

Ruth Ruprecht
Dana Farber Cancer Institute, Boston (US)

John Skehel and Stephen Gamblin
National Institute for Medical Research, The Ridgeway, Mill Hill, London (UK)

Peter Kwong
Vaccine Research Center, Bethesda (US)

Nancy Sullivan
Vaccine Research Center, NIH, Bethesda (US)

Elisa Vicenzi
San Raffaele Scientific Institute, Milan (IT)

Ed Wright and Robin Weiss
University College London (UK)

Giovanna Zambruno
IDI-IRCCS, Rome (IT)

Visiting Scientists

Daniele Lilleri
Fondazione IRCCS Policlinico San Matteo, Pavia (IT)

Joshua Hoong Yu Tan
Oxford University (UK)

Publications

Neutralization and clearance of GM-CSF by autoantibodies in pulmonary alveolar proteinosis.
Luca Piccoli, Ilaria Campo, Chiara Silacci Fregni, Blanca Maria Fernandez Rodriguez, Andrea Minola, Federica Sallusto, Maurizio Luisetti, Davide Corti & Antonio Lanzavecchia
Nat Commun. 2015; 6:7375.

ERK phosphorylation and miR-181a expression modulate activation of human memory T17 cells.
Mele, F., C. Basso, C. Leoni, D. Aschenbrenner, S. Becattini, D. Latorre, A. Lanzavecchia, F. Sallusto and S. Monticelli
Nat Commun. 2015; 6:6431.

Defense-in-depth by mucosally administered anti-HIV dimeric IgA2 and systemic IgG1 mAbs: Complete protection of rhesus monkeys from mucosal SHIV challenge.
Sholukh, A. M., J. D. Watkins, H. K. Vyas, S. Gupta, S. K. Lakhashe, S. Thorat, M. Zhou, G. Hemashettar, B. C. Bachler, D. N. Forthal, F. Villinger, Q. J. Sattentau, R. A. Weiss, G. Agatic, D. Corti, A. Lanzavecchia, J. L. Heeney and R. M. Ruprecht
Vaccine. 2015;

T cell immunity. Functional heterogeneity of human memory CD4(+) T cell clones primed by pathogens or vaccines.
Becattini, S., D. Latorre, F. Mele, M. Foglierini, C. De Gregorio, A. Cassotta, B. Fernandez, S. Kelderman, T. N. Schumacher, D. Corti, A. Lanzavecchia and F. Sallusto
Science. 2015; 347:400-406.

Antibody-driven design of a human cytomegalovirus gHgLpUL128L subunit vaccine that selectively elicits potent neutralizing antibodies.
Kabanova, A., L. Perez, D. Lilleri, J. Marcandalli, G. Agatic, S. Becattini, S. Preite, D. Fuschillo, E. Percivalle, F. Sallusto, G. Gerna, D. Corti and A. Lanzavecchia
Proc Natl Acad Sci U S A. 2014; 111:17965-17970.

Rapid development of broadly influenza neutralizing antibodies through redundant mutations.
Pappas, L., M. Foglierini, L. Piccoli, N. L. Kallewaard, F. Turrini, C. Silacci, B. Fernandez-Rodriguez, G. Agatic, I. Giacchetto-Sasselli, G. Pellicciotta, F. Sallusto, Q. Zhu, E. Vicenzi, D. Corti and A. Lanzavecchia
Nature. 2014; 516:418-422.

Particle Conformation Regulates Antibody Access to a Conserved GII.4 Norovirus Blockade Epitope.
Lindesmith, L. C., E. F. Donaldson, M. Beltramello, S. Pintus, D. Corti, J. Swanstrom, K. Debbink, T. A. Jones, A. Lanzavecchia and R. S. Baric
J Virol. 2014; 88:8826-8842.

Within-Host Evolution Results in Antigenically Distinct GII.4 Noroviruses.
Debbink, K., L. C. Lindesmith, M. T. Ferris, J. Swanstrom, M. Beltramello, D. Corti, A. Lanzavecchia and R. S. Baric
J Virol. 2014; 88:7244-7255.

The Neonatal Fc Receptor (FcRn) Enhances Human Immunodeficiency Virus Type 1 (HIV-1) Transcytosis across Epithelial Cells.
Gupta, S., J. S. Gach, J. C. Becerra, T. B. Phan, J. Pudney, Z. Moldoveanu, S. B. Joseph, G. Landucci, M. J. Supnet, L. H. Ping, D. Corti, B. Moldt, Z. Hel, A. Lanzavecchia, R. M. Ruprecht, D. R. Burton, J. Mestecky, D. J. Anderson and D. N. Forthal
PLoS Pathog. 2013; 9:e1003776.

Cross-neutralization of four paramyxoviruses by a human monoclonal antibody.
Corti, D., S. Bianchi, F. Vanzetta, A. Minola, L. Perez, G. Agatic, B. Guarino, C. Silacci, J. Marcandalli, B. J. Marsland, A. Piralla, E. Percivalle, F. Sallusto, F. Baldanti and A. Lanzavecchia
Nature. 2013; 501:439-443.

Emergence of New Pandemic GII.4 Sydney Norovirus Strain Correlates With Escape From Herd Immunity.
Debbink, K., L. C. Lindesmith, E. F. Donaldson, V. Costantini, M. Beltramello, D. Corti, J. Swanstrom, A. Lanzavecchia, J. Vinje and R. S. Baric
J Infect Dis. 2013; 208:1877-1887.

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; 121:4110-4114.

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
PLoS One. 2013; 8:e59863.

Broadly neutralizing antiviral antibodies.
Corti, D. and A. Lanzavecchia
Annu Rev Immunol. 2013; 31:705-742.

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
Immunity. 2013; 38:596-605.

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.

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.

Lectures and Seminars

2013

Tri-Institutional Immunology and Microbial Pathogenesis Program Research Seminar
Dissecting the human immune response to pathogens and self antigens
New York (US) / 14.01.2013

Keystone Symposium on Emerging Topics in Immune System Plasticity
Human B Cell Memory
Santa Fe (US) / 17.01.2013

Institut Pasteur 8th Meeting of the Club of Vaccinology
HIV neutralizing antibody repertoire for vaccine development
Paris (FR) / 21.01.2013

Keystone Symposium on Antibodies as Drugs
Human Antibodies to Infectious Disease Targets
Vancouver (CA) / 29.01.2013

Karolinska Institute Seminar
Dissecting the human B cell response to pathogens and self antigens
Stockholm (SE) / 14.02.2013

Institut Pasteur 2013 Vaccinology Course Seminar
Immunological memory: the challenge of confering long-term protection?
Paris (FR) / 07.03.2013

Joint Annual Meeting of the Swiss Society for Allergology and Immunology and the Swiss Respiratory Society
New tools and new hopes for improved immune control of infectious diseases
Bern (CH) / 19.04.2013

Joint Annual Meeting of the Swiss Society for Infectious Diseases (SSI), Swiss Society for Hospital Hygiene (SSHH), Swiss Specialists for Tropical and Travel Medicine (SSTTM) and Challenge in HIV-Swiss HIV Cohort Study
Broadly neutralizing antibodies for therapy and vaccine design
Lugano (CH) / 30.05.2013

Charles A. Janeway, Jr., MD Memorial Symposium
The bright and the dark side of somatic mutations
New Haven (US) / 05.06.2013

3rd Annual Meeting of the Laboratory of Excellence IBEID (Integrative Biology of Emerging Infectious Diseases)
Broadly neutralizing antiviral antibodies
Paris (FR) / 21.06.2013

Immunologia: un sistema per la sopravvivenza (conference was part of Festival dei Due Mondi – Spoleto)
Il lato luminoso ed il lato oscuro delle mutazioni somatiche
Spoleto (IT) / 07.07.2013

8th International Symposium on Tonsils and Mucosal Barriers of the Upper Airways (ISTMB) 2013
Broadly neutralizing antiviral antibodies
Zurich (CH) / 18.07.2013

15th International Congress of Immunology 2013
Dissecting the antibody response to pathogens and self antigens
Milan (IT) / 25.08.2013

3rd Basel Immunology Focus Symposium (BIFS3) 2013
The bright and the dark side of somatic mutations
Basel (CH) / 31.08.2013

15th Annual International Meeting of the Institute of Human Virology
Broadly Neutralizing Antibodies for Serotherapy and Vaccine Design
Moscow (RU) / 08.09.2013

The 5th EMBO Meeting 2013
Dissecting the human immune response to pathogens and self antigens
Amsterdam (NL) / 24.09.2013

University of Zurich MD/PhD-MsC Retreat
Dissecting the antibody response to pathogens and self antigens
Ascona (CH) / 06.10.2013

Keystone Symposium on Advancing Vaccines in the Genomics Era
Dissecting the antibody response to pathogens and self antigens
Rio de Janeiro (BR) / 02.11.2013

BSM 2013: 3rd ESOT Basic Science Meeting and 13th TTS Basic Science Symposium
Cellular Basis of Immunologic Memory
Paris (FR) / 07.11.2013

Dimet Course on Molecular Immunology - INGM
B lymphocytes and T lymphocytes (2 different seminars on same day)
Milan (IT) / 14.11.2013

Cold Spring Harbor Symposium on Harnessing Immunity to Prevent and Treat Disease
Analytic vaccinology
Cold Spring Harbor (US) / 22.11.2013

Lecture at Centre for Immune Regulation – University of Oslo
Dissecting the human antibody response to pathogens and self antigens
Oslo (NO) / 13.12.2013

Lectures and Seminars

2014

Keystone Symposium on Biology of B Cell Responses
B Cell Memory to Infection
Keystone (US) / 11.02.2014

Second International Course on Translational Hepatology: The HCV And HBV Diseases – A View From The Bridge Between The Bench And The Bedside
Dissecting the human immune response to pathogens and self antigens
Florence (IT) / 10.03.2014

10th Spring School on Immunology
Dissecting the human B and T cell response to pathogens and self antigens
Ettal (DE) / 14.03.2014

Conference Kiwanis Club Lugano
Gli anticorpi come farmaci tuttofare
Lugano (CH) / 24.03.2014

Conference Rotary Club Locarno
Ricerca Biomedica: Vaccini e Nuove Terapie
Locarno (CH) / 01.04.2014

University of Pittsburgh 2014 Eberly Distinguished Lectureship in Immunology
Dissecting the Human Antibody Response to Pathogens and Self Antigens
Pittsburgh (US) / 03.04.2014

EASL (European Association for the Study of the Liver) – The International Liver Congress 2014
Recombinant Human Neutralizing Antibodies as Future Antiviral Agents
London (UK) / 12.04.2014

Seminar at The Peter Doherty Institute
The bright and the dark side of somatic mutations
Melbourne (AU) / 06.05.2014

5th Australian Vaccines & Immunotherapeutics Development Meeting (AVID 2014)
Opening plenary lecture (no title)
Melbourne (AU) / 07.05.2014

Collegio Ghislieri 13° corso di formazione avanzata: Nuovi bersagli di terapia cellulare
Immuno-sorveglianza e immuno-tolleranza
Pavia (IT) / 12.05.2014

Be the Cure Tolerance Workshop
B cell immunity to autoantigens and microbes analysed at the single cell level
Sigtuna (SE) / 24.05.2014

Gordon Research Conference on Immunochemistry & Immunobiology
Affinity Maturation of Broadly Neutralizing Antibodies
Newry (US) / 04.06.2014

Graduate Schools Forum on Infection & Immunity
Dissecting the human immune response to pathogens
Geneva (CH) / 06.06.2014

Seminar at Arabian Gulf University
Passive vaccination for epidemic and emerging respiratory viruses
Manama (BH) / 17.06.2014

20th International AIDS Conference 2014
Stepping up the Pace on HIV Vaccine: What Needs to Be Done?
Melbourne (AU) / 24.07.2014

Winter Seminar of the Immunology Group of Victoria
The bright and the dark side of somatic mutations
Melbourne (AU) / 25.07.2014

16th International Congress of Virology (IUMS 2014)
Dissecting the antibody response to pathogens
Montreal (CA) / 01.08.2014

From the Laboratory to the Clinic Meeting: Translating Imaging and Other Novel Approaches
The bright and the dark side of somatic mutations
Oxford (UK) / 17.09.2014

20th Training Course of the Swiss Society of Hematology
Physiology of plasma cells
Zurich (CH) / 24.10.2014

China Tregs/Th Subsets 2014 Conference
Dissecting the human immune response to pathogens and vaccines
Shanghai (CN) / 04.11.2014

12th International Congress of Neuroimmunology (ISNI)
On the Origin of Autoantibodies
Mainz (DE) / 13.11.2014

11th Annual Meeting of the Society for Melanoma Research (2014 SMR Congress)
The bright and the dark side of somatic mutations
Zurich (CH) / 15.11.2014

DKF Research Conference (University of Bern, Department of Clinical Research Conference)
Dissecting the antibody response to pathogens and self antigens
Bern (CH) / 01.12.2014

43rd Annual Meeting of the Japanese Society for Immunology
The bright and the dark side of somatic mutations
Kyoto (JP) / 11.12.2014

Human Immunology Forum 2014
Dissecting the antibody response to pathogens and self antigens
Kyoto (JP) / 12.12.2014

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 endoplasmic reticulum associated degradation (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, the Research Award Aetas 2007 and the Regli Foundation Award 2013. 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 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 identify drug targets and/or to design therapies for diseases caused by inefficient functioning of the cellular protein factory, resulting from expression of defective gene products (e.g., rare genetic disorders), or elicited by pathogens.

Team

Group Leader: Maurizio Molinari, PhD > maurizio.molinari@irb.usi.ch

Members: Eduardo Cebollero, PhD - Giorgia Brambilla Pisoni, PhD student - Elisa Fasana, PhD - Carmela Galli Molinari, MSc - Ilaria Fregno, PhD student - Tatiana Soldà, MSc - Timothy Jan Bergmann, PhD student - Fiorenza Fumagalli, PhD student.

Substrate-Specific Mechanisms of Protein Degradation from the ER

Giorgia Brambilla Pisoni, Timothy Jan Bergmann, Ilaria Fregno, Elisa Fasana, 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 defined as 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, whose fate will be monitored in mammalian cultured cells. The model polypeptides recapitulate structural defects found in mutant products of genes causing human disorders such as Alzheimer's, Parkinson's, Huntington's diseases as well as many other rare genetic disorders characterized by gain-of-toxic-function or loss-of-function phenotypes. How the polypeptide's features determine engagement of specific folding, quality control and degradation pathways will be determined in molecular details.

* ***Merulla J. et al***
Traffic. 2013, 14:767-777.

Comparative Interactomics to Identify Novel ER-Resident Quality Control Players

Giorgia Brambilla Pisoni, 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 collection of 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.

Novel Protein Quality Checkpoints

Ilaria Fregno, Tatiana Soldà and Maurizio Molinari

Proteins that reach the native structure are released from the ER and are transported to their site of activity. Misfolded polypeptides are selected for degradation. The stringency of protein quality control in the mammalian ER may lead to the removal of *structural-defective* polypeptides, independent of their capacity to fulfill their *function*. This causes loss-of-function proteopathies such as cystic fibrosis, lysosomal storage dis-

eases and many others, where functional polypeptides are inappropriately removed from cells because they display minor structural defects. The development of therapeutic strategies to treat such disorders relies on the characterization, at the molecular level, of the quality checkpoints and pathways operating in mammalian cells. Recently published data in our group show that proteins with native ectodomains presenting an intramembrane defect (an ionizable residue in the transmembrane domain spanning the lipid bilayer) alert a novel post-ER quality control and are retained in a pre-Golgi compartment. This novel checkpoint involves the cytosolic AAA-ATPase p97 and the luminal factor UDP-glucose:glycoprotein glucosyltransferase (UGGT1) and can be by-passed, thus resulting in surface transport of the defective protein, upon p97 inhibition or p97 and UGGT1 silencing (Figure 1). To better characterize this novel protein quality control machinery, we generated mammalian tetracycline-inducible cell lines individually expressing two type I membrane protein chimeras. The first consists in the folding competent ectodomain of human α1-antitrypsin fused with the C-terminal domain of CD3δ, which contains an ionizable aspartic acid at position 6 in the intramembrane sequence (chimera α1ATc). The second in α1-antitrypsin fused with the same domain where the ionizable residue is replaced with an alanine (chimera $\alpha1ATc_{D6A}$). Ongoing work is focused on the identification of the components of this p97/UGGT1-mediated checkpoint.

***Ferris S. P. et al.** **
Mol Biol Cell. 2013, 24:2597-2608.

***Merulla J. et al.** **
Mol Biol Cell. 2015, 26:1532-1542.

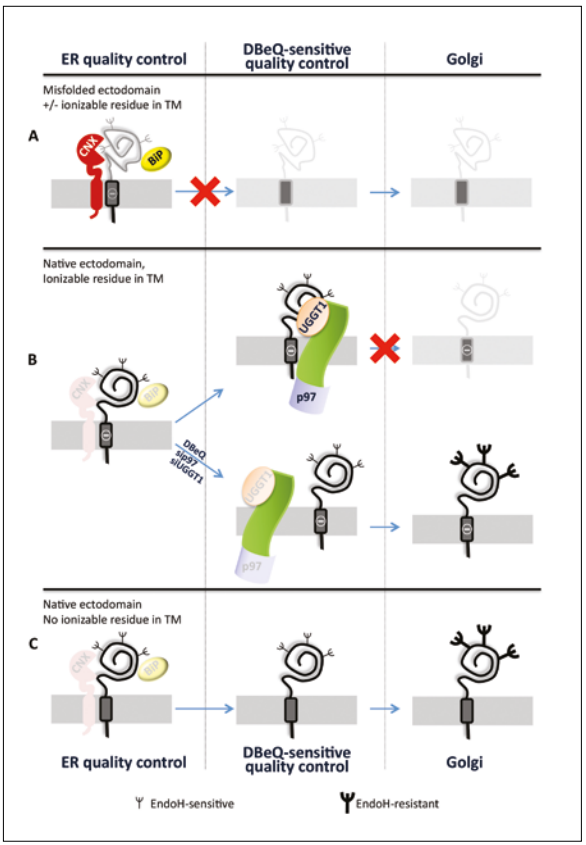


Figure 1
A novel retention-based quality checkpoint.
(A) Proteins with a misfolded ectodomain are retained in the ER by the conventional quality control relying on UGGT1, CNX and BiP intervention. These proteins are eventually destined to ERAD. (B) A chimera with a native ectodomain, characterized by an ionizable residue in the intramembrane domain (α1ATc) fulfills quality control requirement for release from CNX and BiP, but its transport to the Golgi is halted upon p97 and UGGT1 intervention. This protein quality checkpoint is by-passed upon pharmacologic inhibition of p97 with DBeQ or upon silencing of p97 or UGGT1 expression. (C) Replacement of the ionizable intramembrane residue with an alanine results in efficient transport to the Golgi compartment.

Disposal of Non-Glycosylated Polypeptides from the Mammalian ER

Timothy Jan Bergmann, Elisa Fasana, Tatiana Soldà and Maurizio Molinari

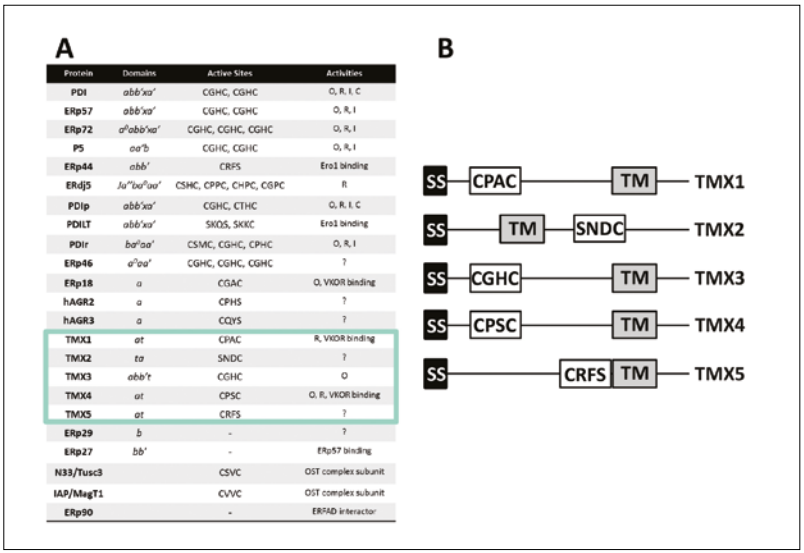
To maintain ER homeostasis and to ensure the highest efficiency of functional polypeptide production, the quality control machinery operating in the mammalian ER must distinguish non-native intermediates of protein folding programs from terminally misfolded polypeptides. Folding intermediates must be retained in the ER to attain the native structure under the assistance of dedicated molecular chaperones and folding enzymes. Terminally misfolded polypeptides must be 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.

* ***Tannous A. et al.***
Semin Cell Dev Biol. 2015, in press

Role of Membrane-Bound Oxidoreductases in Protein Biogenesis

Giorgia Brambilla Pisoni and Maurizio Molinari

The lumen of the ER contains 23 PDI members that insure formation of the correct set of intra- and intermolecular disulfide bonds as a crucial, rate-limiting reaction of the protein folding process (Figure 2A). The reason for this high redundancy of PDIs remains unclear. Certainly, individual members of the PDI family show tissue-specific distribution or some kind of substrate preference (e.g. ERp57 forms functional complexes with the ER lectins calnexin and calreticulin and acts upon their ligands). The aim of this project is to uncover the role in protein biogenesis of the 5 type I membrane-bound members of the PDI family (TMX1, TMX2, TMX3, TMX4 and TMX5) (Figure 2B). Active PDIs contain the characteristic CXXC active-site motif that engages folding substrates in so-called mixed disulfides (i.e. covalent bonds between a PDI and a substrate cysteine). Mixed disulfides are extremely short living intermediates of the protein folding reaction, which can be stabilized upon replacement of the second (resolving) cysteine residue in the PDIs catalytic site. These so-called PDIs "trapping mutants" have been used to capture endogenous substrates of select ER-resident oxidoreductases such as ERp57, PDI, P5, ERp18, ERp72, ERp46 and ERdj5. The expression of a TMX1 trapping mutant in the living cells and the characterization by mass spectrometry of the polypeptides remaining covalently bound to it revealed a selective association with a series of cysteine-containing membrane-bound proteins. This is in contrast to studies performed with trapping mutants of other PDIs, which were all found to associate both with soluble and membrane-bound endogenous substrates. Studies are ongoing to confirm the substrate topology-dependent specificity of TMX1 and to characterize the role in protein biogenesis of the other TMX proteins (Figure 3).



Protein	Domains	Active Sites	Activities
PDI	abb'xa'	CGHC, CGHC	O, R, I, C
ERp57	abb'xa'	CGHC, CGHC	O, R, I
ERp72	$a^0abb'xa'$	CGHC, CGHC, CGHC	O, R, I
P5	$aa'b$	CGHC, CGHC	O, R, I
ERp44	abb'	CRFS	Ero1 binding
ERdj5	$Ja''ix^0ua'$	CSHC, CPPC, CHPC, CGPC	ft
PDIp	abb'xa'	CGHC, CTHC	O, R, I, C
PDILT	abb'xa'	SKQS, SKKC	Ero1 binding
PDIr	ba^0aa'	CSMC, CGHC, CPHC	O, R, I
ERp46	a^0aa'	CGHC, CGHC, CGHC	?
ERp18	a	CGAC	O, VKOR binding
hAGR2	a	CPHS	?
hAGR3	a	CQYS	?
TMX1	at	CPAC	R, VKOR binding
TMX2	ta	SNDC	?
TMX3	abb't	CGHC	O
TMX4	at	CPSC	O, R, VKOR binding
TMX5	at	CRFS	?
ERp29	b	-	?
ERp27	bb'	-	ERp57 binding
N33/Tusc3		CSVC	OST complex subunit
IAP/MagT1		CVVC	OST complex subunit
ERp90		-	ERFAD interactor

Figure 2
The PDI family.
(A) The PDI family comprises 23 members. The 5 type I membrane-bound PDI family members (TMX proteins) are highlighted. (B) Domain structure of TMX1-TMX5.

Figure 3
PDIs interactors.
Endogenous proteins associated with the ERdj5 (Oka et al. 2013) and TMX1 trapping mutant are shown.

Interactors of ERdj5

ER-Resident Proteins	Membrane Proteins	Secreted Proteins
Peroxiredoxin-4	LDLR	EGF-containing fibulin-like protein 1
BiP	LDLR-related protein	Laminin-5 beta3
P5	Death receptor 5	TGF-β
ERp72	Integrin beta-1A	Fibronectin
ERp57	Amyloid-beta protein	Laminin subunit gamma
PDI	MUC18 glycoprotein	Collagen alpha-3(VI)
Ero1	NOTCH 2	Stanniocalcin-1
Erp44	Attractin-2	Laminin B2
UGT1	Mannose 6-phosphate receptor	Laminin B1
ERp46	EGFR	Agrin
Glucosidase II beta subunit		Pentaxin
Grp94		TGF-β binding protein
		Collagen alpha-1(VI)
		Collagen alpha-2(IV)

Interactors of TMX1

ER-Resident Proteins	Membrane Proteins	Secreted Proteins
BiP	Integrin beta-1	
ERp57	Slc3a2	
CNX	Ece1	
Erp44	Lrrc59	
Uggt1	Nicastrin	
	Atp6ap1	
	Tspan3	
	Lrp10	
	Pld4	

Cellular Responses to Variations in ER Homeostasis and Protein Load

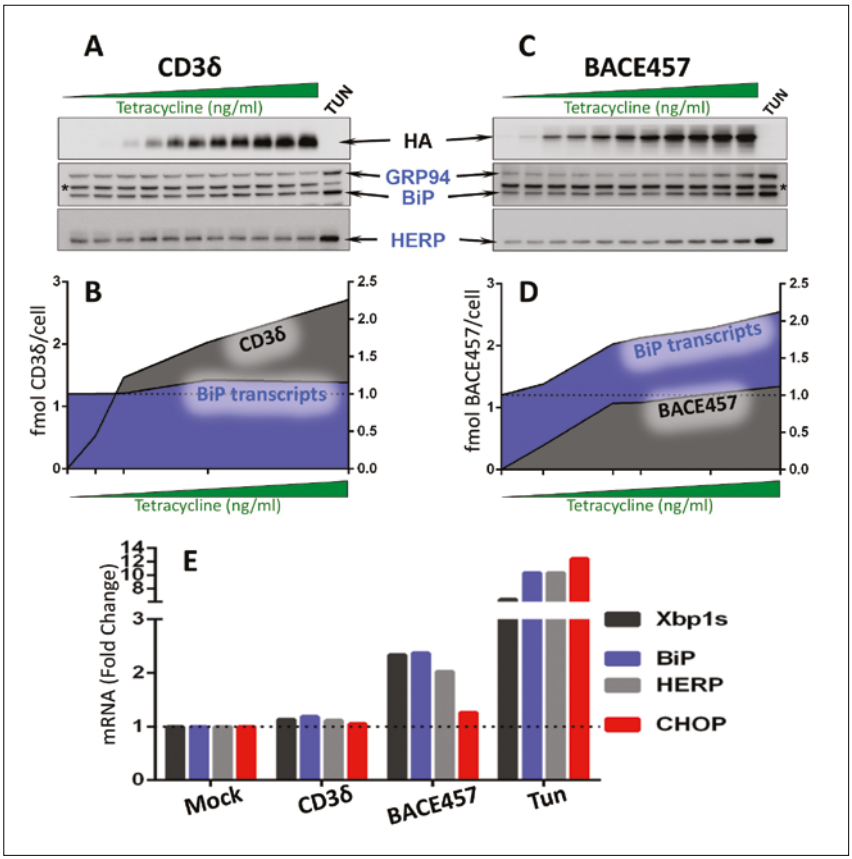
Timothy Jan Bergmann, Ilaria Fregno, Carmela Galli and Maurizio Molinari

The equilibrium between protein synthesis, export and ERAD is crucial for maintaining ER homeostasis. Different physiological and pathological conditions (e.g. fluctuations in protein synthesis, accumulation of defective gene products, pathogens…) can perturb the ER environment, leading to conditions of ER-stress. Such stresses can lead to the activation of the UPR, adaptive, transcriptional and translational programs that induce the expression of ER resident gene products, increase the ER size and reduce synthesis of cargo protein in order to restore ER homeostasis. The aim of this project is to investigate how cells respond to perturbations of the ER environment upon tunable expression of a selection of model proteins with different physico-chemical features or upon exposure to ER-stress inducing drugs. Experimental data from the lab show that cells respond differently to increasing amount of misfolded ER proteins. While some proteins do not induce an UPR even at high molar concentrations (Figure 4A-4B, 4E), other proteins elicit UPR already at low dosage (Figure 4C-4E). Thus, the threshold for activation of transcriptional programs in response to increasing burden of misfolded protein (Figure 5) must depend on intrinsic features of the accumulating polypeptide. We will couple genome wide gene expression profiling (in collaboration with F. Bertoni, IOR, Bellinzona) with proteome analysis, shotgun and selected reaction monitoring-based (SRM) proteomics (in collaboration with P. Picotti, ETH Zurich) in order to establish transcriptional and post-translational cellular response "fingerprints" associated to individual defective polypeptide expression and drug treatments. Responses to accumulation of misfolded proteins below the threshold required for UPR activation will be analyzed with particular care. These responses that we collectively termed ERAD tuning could rely on post-translational mechanisms, which have much shorter latency, since they do not depend on gene transcription and translation (e.g. modulation of ER-resident proteins turnover, formation/disassembly of functional complexes, sub-compartmental de-localization, post-translational modifications such as ADP-ribosylation, palmitoylation, …). Some of these non-transcriptional responses that regulate ER-resident proteins level and activity are hijacked by human pathogens during their infection cycle.

* ***Noack J. et al.***
J Virol. 2014, 88:10272-10275.

* ***Noack J. et al.***
Nat Chem Biol. 2014, 10:881-882.

* ***Noack J. et al.***
Swiss Med Wkly. 2014, 144:w14001.



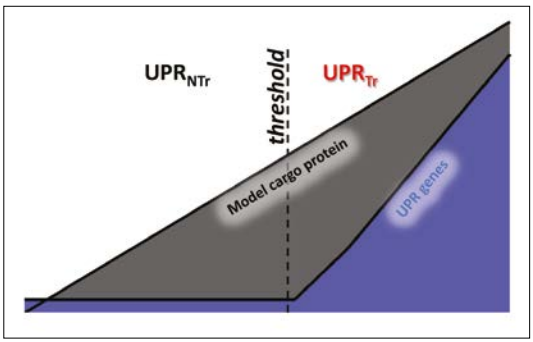


Figure 4
Substrate-dependent ER stress-induction.
(A) Tet-induced expression of CD3δ-HA. BiP, GRP94 and HERP levels. Last lane, Tun-induced ER stress (5μg/ml). (B) Variations of CD3δ (fmol protein/ cell) and BiP transcripts. **(C)-(D)** *same as* **(A)-(B)** *for BACE457. (E) Induction of stress markers at maximal CD3δ and BACE457 doses and at 5μg/ml Tun, quantitative real time PCR*

Figure 5
Induction of non-transcriptional and transcriptional UPR.
Threshold (dotted line) shows the level of model protein that triggers a transcriptional UPR (UPR$_{Tr}$). Below the threshold, responses, if any, are not-transcriptional, and ill-defined

The Function and Regulation of ER-Phagy During ER Homeostasis and Stress

Eduardo Cebollero, Fiorenza Fumagalli, Carmela Galli and Maurizio Molinari

Autophagy is a conserved cellular process in eukaryotes required for degradation of cytoplasm contents into the lysosome/vacuole. Double-membrane vesicles called autophagosomes mediate the engulfment and transport of the cargo to be degraded during autophagy. While this pathway constitutively degrades cytoplasmic targets, it is also up-regulated by different cellular stresses. Starvation-induced autophagy randomly targets bulk cytoplasmic portions. Additionally, it selectively recognizes and degrades cytoplasmic protein aggregates, damaged organelles or invading microorganisms, playing thus a homeostatic and protective role in the cell. Interestingly, accumulation of misfolded proteins within the ER triggers autophagic degradation of portions of this organelle in yeast and mammals, suggesting that ER-phagy might be a conserved mechanism to prevent or overcome ER stress. While ERAD pathway is the classical and best characterized process for protein disposal in the ER, little is known about the mechanisms underlying ER degradation by autophagy. By using series of stable human cell lines created in our lab expressing regulated amounts of folding-competent and folding-defective protein chimeras, we are studying the contribution of autophagy in the degradation of these putative substrates and the molecular mechanisms regulating such a process. These studies will allow us to characterize the conditions for potential preferences in substrate elimination by ERAD and ER-phagy, and the mechanistic crosstalk between these two pathways and ER stress. The information generated by these studies will be validated in pathological model systems expressing disease-causing folding-defective proteins with the final goal of designing pharmacological treatments targeting protein disposal pathways to alleviate the toxicity caused by aberrant protein accumulation.

Revealing Mechanisms Regulating Recovery from Transient ER Stress in Mammalian Cells

Fiorenza Fumagalli, Eduardo Cebollero, Carmela Galli and Maurizio Molinari

Eukaryotic cells respond to changes in ER homeostasis by reducing the synthesis of cargo proteins, by inducing transcription/translation of ER-resident gene products and by expanding the ER volume in a series of events collectively named the UPR. The *temporary* reduction in cargo protein synthesis coupled with the enhanced luminal content of molecular chaperones, folding and ERAD factors should reduce the burden of unfolded and misfolded polypeptides in the ER lumen and re-establish proteostasis (i.e., the capacity to produce the functional cellular proteome in appropriate amount). Most studies have focused on transcriptional events and their regulation during UPR elicited by drugs that dramatically impair cellular (and not only ER) homeostasis by compromising the regulation of redox conditions, calcium concentration or protein glycosylation.

The aim of this project is to establish experimental conditions resulting in triggering reversible ER stresses. To this end, cells will be transiently challenged with different drugs perturbing compartmental homeostasis. Alternatively, cell lines will be generated, which are characterized by inducible, tetracycline-controlled expression of a collection of folding-defective polypeptides. The model proteins will be individually expressed at levels triggering an UPR and their synthesis will then be stopped upon tetracycline wash out. How do cells return to the "steady state situation" (i.e. how the excess chaperones produced during the stress phase is cleared from cells during the "recovery phase" occurring after ER stress resolution (Figure 6) is the main subject of our study.

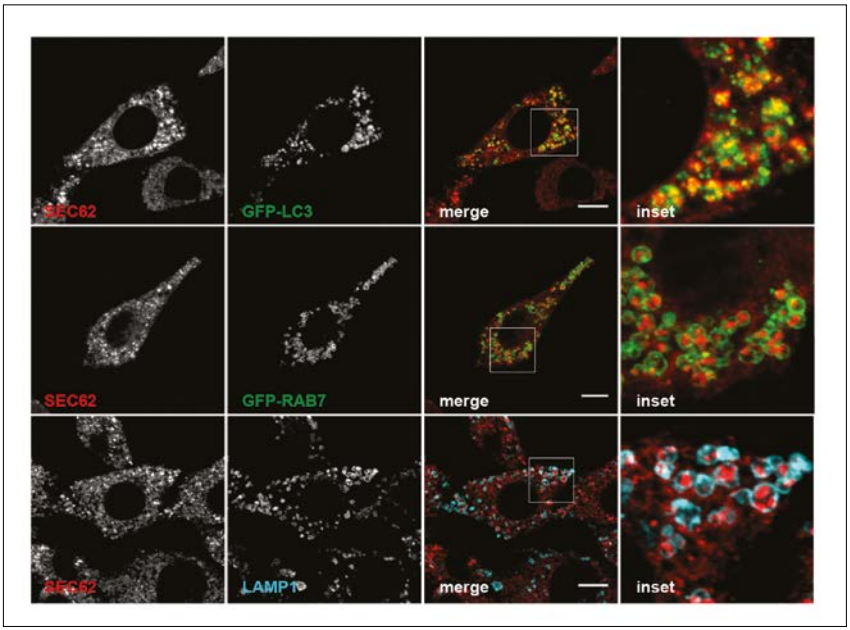


Figure 6
SEC62 accumulates in autophagic vacuoles upon lysosomal inhibition during stress recovery.
Mouse embryonic fibroblasts transfected or not with GFP-LC3 (upper panels) or GFP-RAB7 (middle panels) were treated with 50 nM BafA1 during 12 h of stress recovery. Immunostains are with anti-SEC62 (all panels) and anti-LAMP1 (lower panels).

Generating Antibodies to Contrast Aβ Deposition

Ilaria Fregno, Tatiana Soldà and Maurizio Molinari

***Paganetti P. et al.* ***
Neurobiol of Aging. 2013; 34:2866-2878.

We engineered antibodies to be expressed in a transgenic mice model for Alzheimer's disease from a single open reading frame encoding the heavy and light chain of a specific antibody to the N-terminus of the Aβ peptide linked by the mouth and foot virus peptide 2A (to promote equimolar expression of light and heavy chains that are efficiently processed and assembled in the ER of transgenic cells). The *in situ* production of the antibody substantially reduced Aβ formation and aggregation compared to age-matched, mock-treated APP23 mice.

Funding

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

Swiss National Science Foundation Sinergia
ER-phagy mechanisms to maintain and restore ER homeostasis
CRSII3-154421

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

Gelu Foundation
Studies on conformational diseases of the elderly and the children

Signora Alessandra
Investigating the mechanisms regulating native proteins production

Fondazione Comel
Studies on conformational proteopathies

Collaborations

Fulvio Reggiori
University Medical Center Utrecht (NL)

Manfredo Quadroni
University of Lausanne (CH)

Paola Picotti
ETH-Zurich (CH)

Neil Bulleid
University of Glasgow (UK)

Lloyd Ruddock
University of Oulu (FI)

Visiting Scientists

Ivan Hang
August 2013
ETH-Zurich (CH)

Ilaria Fregno
March-October 2014 (Erasmus)
University of Padua (IT)

Alessio Nembrini
May 2014
Liceo Bellinzona (CH)

Publications

Glycoprotein maturation and quality control.
M. Molinari and D. N. Hebert
Semin Cell Dev Biol. 2015; in press

A novel UGGT1 and p97-dependent checkpoint for native ectodomains with ionizable intramembrane residue.
Merulla, J., T. Solda and M. Molinari
Mol Biol Cell. 2015; 26:1532-1542.

N-linked sugar-regulated protein folding and quality control in the ER.
Tannous, A., G. B. Pisoni, D. N. Hebert and M. Molinari
Semin Cell Dev Biol. 2015; in press

Protein trafficking: RESETting proteostasis.
Noack, J. and M. Molinari
Nat Chem Biol. 2014; 10:881-882.

Proteostasis: Bad news and good news from the endoplasmic reticulum.
Noack, J., G. Brambilla Pisoni and M. Molinari
Swiss Med Wkly. 2014; 144:w14001.

How viruses hijack the ERAD tuning machinery.
Noack, J., R. Bernasconi and M. Molinari
J Virol. 2014; 88:10272-10275.

Transgenic expression of beta1 antibody in brain neurons impairs age-dependent amyloid deposition in APP23 mice.
Paganetti, P., J. Reichwald, D. Bleckmann, D. Abramowski, D. Ammaturo, C. Barske, S. Danner, M. Molinari, M. Muller, S. Papin, S. Rabe, P. Schmid and M. Staufenbiel
Neurobiol Aging. 2013; 34:2866-2878.

UDP-glucose:glycoprotein glucosyltransferase (UGGT1) promotes substrate solubility in the endoplasmic reticulum.
Ferris, S. P., N. S. Jaber, M. Molinari, P. Arvan and R. J. Kaufman
Mol Biol Cell. 2013; 24:2597-2608.

Specificity and regulation of the endoplasmic reticulum-associated degradation machinery.
Merulla, J., E. Fasana, T. Solda and M. Molinari
Traffic. 2013; 14:767-777.

Lectures and Seminars

Engineers Shape our Future 2013, Il Mondo delle Tecnologie
La Ricerca Biomedica in Ticino
Locarno (CH) / 21.01.2013

Protein folding and quality control in the living cell
Institute of Computational Science, Lugano (CH) / 03.04.2013

EMBO Conference on Protein Transport
ERAD tuning: setting the luminal activity of ERAD factors by regulating their segregation and/or turnover
Dubrovnik (HR) / 13-17.04.2013

The ER and Redox Club Meeting
Regulation of ER-associated degradation
Warwick (UK) / 25-27.04.2013

FASEB Summer Research Conference "From Unfolded Proteins in the Endoplasmic Reticulum to Disease"
ERAD substrate-dependent regulation of ER-associated degradation
Saxtons River, Vermont (US) / 16-21.06.2013

Basic Virology Course
From How Viruses Hijack Host Cell Machineries … to … How Viruses Help us in Deciphering Protein Biogenesis
Institut Pasteur, Paris (FR) / 03.09.2013

Meet the Expert
Ripiegamento di Proteine e Proteopatie
Liceo Lugano 1, Lugano (CH) / 22.11.2013

Meet the Expert
Ripiegamento di Proteine e Proteopatie
Liceo Lugano 1, Lugano (CH) / 24.02.2014

The ER and Redox Club Meeting
Division of Labor between Oxidoreductases
Venezia (IT) / 25-27.04.2015

11th International Calreticulin Workshop
A Novel UGGT1- and p97-Dependent Checkpoint for Native Ectodomain with Ionizable Intramembrane Residue
New York (US) / 15-18.05.2015

Organization of international congresses

Eleventh International Calreticulin Workshop, New York (US) / 15-18.05.2015
(http://www.crt2015.com/#!organizing-committee/c1pf8)

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 Center for Blood Research, Harvard Medical School in Boston (US), where she continued her scientific training by performing studies aimed to understand the mechanisms of regulation of cytokine transcription in T lymphocytes and mast cells. In 2007 she joined the Institute for Research in Biomedicine in Bellinzona as Group Leader. Silvia Monticelli has published several papers covering various aspects of immunological processes, with a special focus on the regulation and function of T lymphocytes and mast cells. Her major research interests are focused on the role of regulatory microRNAs as well as epigenetic modifications in the activation and function of cells of the immune system.

Research Focus

Our lab is interested in understanding epigenetic mechanisms of regulation of gene expression, which might be important for the development of a number of immunological diseases, from neoplasia of mast cells (mastocytosis) to autoinflammatory disorders. Epigenetic inheritance is usually independent from the DNA sequence encoding a given gene, and while in the most stringent definition this includes mostly DNA methylation (and its derivatives), it can also more broadly include histone modifications and even microRNAs (miRNAs). Our lab is mostly interested in understanding the role of DNA methylation dynamics in regulating cell differentiation and function, as well as the interplay between the DNA methylation machinery and miRNA expression. MiRNAs are 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 both DNA methylation and 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 malignancies and autoinflammatory diseases.

Team

Group Leader: Silvia Monticelli, PhD > silvia.monticelli@irb.usi.ch
Members: Lorenzo Dehò, specialization student - Stefan Emming, PhD student - Cristina Leoni, PhD student - Sara Montagner, PhD student - Lucia Vincenzetti, PhD student

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

Sara Montagner, Cristina Leoni, Lorenzo Dehò and Silvia Monticelli

Systemic mastocytosis is a rare neoplasm due to the abnormal accumulation and infiltration of mast cells in various organs and tissues. The prognosis for this disease 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, but the factors that contribute to mastocytosis variants are still largely obscure. We are investigating alterations of miRNA regulation and abnormal patters of DNA methylation that may lead to epigenetic modifications of gene expression in patients, and ultimately to tumorigenic transformation (Montagner S., Immunol Rev., 2013). In particular, we are interested in understanding the role of DNA methylation and hydroxymethylation in mast cell functions and mast cell-related diseases. We investigated changes in DNA methylation (5mC) and hydroxymethylation (5hmC) in patients with systemic mastocytosis, and we found that overall 5hmC levels were reduced in all patients with systemic mastocytosis, but to a greater extent in the presence of higher D816V mutational load in the KIT oncogene, which affects prognosis and therapeutic options in these patients (Leoni C., Eur J Haematol., 2015). These findings may potentially become useful for patient stratification and possibly even prognosis. Currently, we are investigating the role of Tet2, a DNA-modifying enzyme responsible for cytosine hydroxymethylation, in mast cell biology (Figure 1).

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

* ***Leoni C et al.***
Eur J Haematol. 2015, in Press

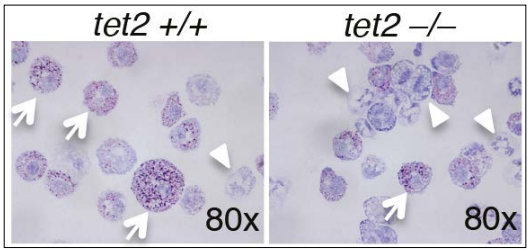


Figure 1
Altered mast cell differentiation in the absence of Tet2.
Toluidine blue staining showing abundance of mast cells (arrows) in normal cells (+/+), and presence of other types of myeloid cells (arrowheads) when the DNA-modifying enzyme Tet2 is deleted (-/-) (Montagner S. unpublished data).

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

Lorenzo Dehò, Cristina Leoni, Sara Montagner and Silvia Monticelli

Dehò L et al*
J Immunol. 2014, 193:2196-2206.

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 identified 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 (Figure 2) (Dehò L., J Immunol., 2014).

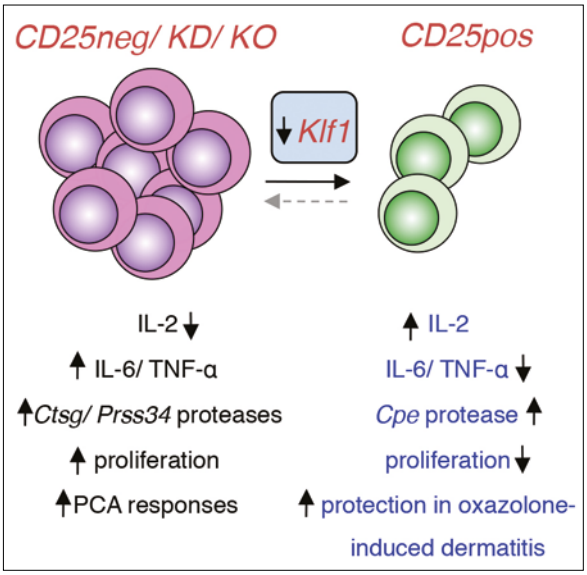


Figure 2
Two novel mast cell subsets identified by CD25 expression.
Each subset is characterized by unique abilities to proliferate, express cytokines and to contribute to disease in models of mast cell-related pathologies (passive cutaneous anaphylaxis and oxazolone-induced dermatitis) (Dehò L et al. J Immunol 2014).

Transcription factors and miRNAs in the regulation of human T_H17 lymphocytes

Federico Mele, Cristina Leoni, Stefan Emming, 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 we identified a novel molecular network comprising transcription factors and miRNAs involved in the modulation of T cell receptor signal strength specifically in Th17 cells (Figure 3) (Mele F., Nat Commun., 2015). We are now continuing to investigate the role of a number of miRNAs in the regulation of T cell activation, metabolism and plasticity.

*** *Mele F. et al.***
Nat Commun. 2015, 6:6431.

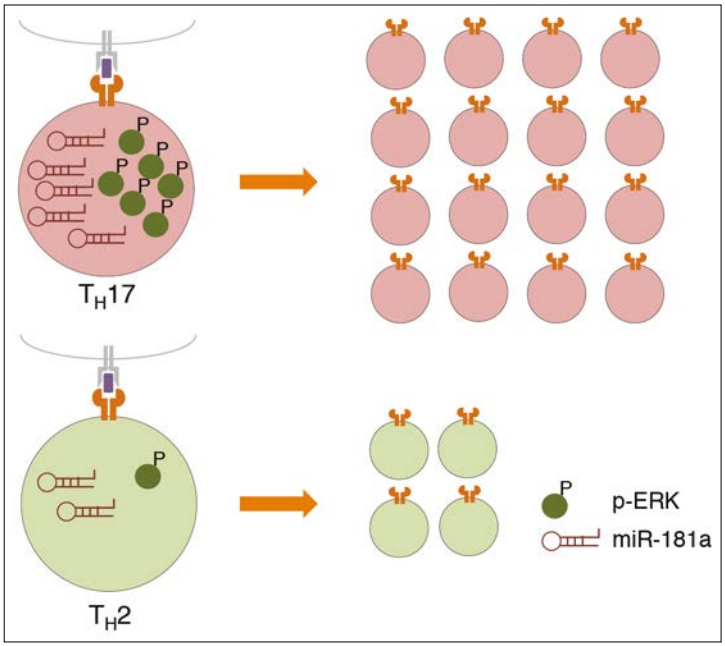


Figure 3
Role of miR-181a in regulating T cell activation.
Given the same T cell receptor, memory T cells with higher miR-181a expression will respond with overt proliferation to lower concentrations of antigen, at least in part by modulating levels of phosphorylated ERK. (Mele F. et al. Nat Commun., 2015)

DNA methylation dynamics in the functional regulation of human T lymphocytes

Lucia Vincenzetti and Silvia Monticelli

Efficient immune responses orchestrated by CD4 T lymphocytes require both lineage commitment and phenotypic flexibility, allowing the development of responses tailored to invading pathogens. With this project we aim at comprehensively investigating the role of DNA modifications and DNA-modifying enzymes in human T cell responses. Specifically, we want to address fundamental questions about the gene regulatory networks controlling the balance between commitment, phenotypic stability and plasticity of T cells. This will be performed by combining genome-wide analyses of DNA modifications and genetic manipulation of primary human T cells. Indeed, the stability of DNA methylation and its heritability across mitosis make it particularly apt to mediate the maintenance of transcriptional networks and cellular phenotypes. In the case of T cells, however, stability in the expression of subset-selective genes (notably cytokine genes) must be reconciled with mechanisms enabling plastic phenotypic changes in response to environmental clues. The recent discovery that methylated DNA can be dynamically modified, impacting gene expression directly or via erasure of DNA methylation, suggests its possible role in T cell plasticity. Our study will thoroughly describe dynamics in the methylation landscape in primary human T cells in response to specific pathogens and antigens, and assess the effects of methylation dynamics in T cell functions, leading to novel insights in immunity against pathogens and in disease.

Funding

Swiss National Science Foundation
DNA methylation landscape in mast cell differentiation and systemic mastocytosis
31003A_156875 / 2015-2018

Ceresio Foundation
Fellowship contribution towards the salary of one PhD student / 2015-2018

Swiss Multiple Sclerosis Society
Role of miRNAs in the regulation of human T cell activation and in the development of EAE / 2014

San Salvatore Foundation for Cancer Research
Identification of novel genetic and epigenetic determinants for oncogenic transformation in patients with systemic mastocytosis / 2013-2015

Novartis Stiftung für Medizinisch Biologische Forschung
Role of 5'-hydroxymethylcytosine epigenetic modification in a myeloproliferative disorder / 2013

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

Collaborations

Federica Sallusto
Institute for Research in Biomedicine, Bellinzona (CH)

Gioacchino Natoli
European Institute of Oncology, Milan (IT)

Publications

ERK phosphorylation and miR-181a expression modulate activation of human memory T17 cells
Mele, F., C. Basso, C. Leoni, D. Aschenbrenner, S. Becattini, D. Latorre, A. Lanzavecchia, F. Sallusto and S. Monticelli
Nat Commun. 2015; 6:6431.

Reduced DNA methylation and hydroxymethylation in patients with systemic mastocytosis.
Leoni, C., S. Montagner, L. Deho, R. D'Antuono, G. Matteis, A. V. Marzano, S. Merante, E. M. Orlandi, R. Zanotti and S. Monticelli
Eur J Haematol. 2015; in Press

Two functionally distinct subsets of mast cells discriminated By IL-2-independent CD25 activities.
Deho, L., C. Leoni, T. M. Brodie, S. Montagner, M. De Simone, S. Polletti, I. Barozzi, G. Natoli and S. Monticelli
J Immunol. 2014; 193:2196-2206.

MicroRNAs in hematopoietic development.
Montagner, S., L. Deho and S. Monticelli
BMC Immunol. 2014; 15:14.

Macrophage activation: glancing into diversity.
Natoli, G. and S. Monticelli
Immunity. 2014; 40:175-177.

The many faces of CD4 T cells: Roles in immunity and disease.
Sallusto, F. and S. Monticelli
Semin Immunol. 2013; 25:249-251.

MicroRNAs in T helper cell differentiation and plasticity.
Monticelli, S.
Semin Immunol. 2013; 25:291-298.

Short-term memory of danger signals and environmental stimuli in immune cells.
Monticelli, S. and G. Natoli
Nat Immunol. 2013; 14:777-784.

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.

Lectures and Seminars

World Immune Regulation Meeting VII, "Innate and Adaptive Immune Response and Role of Tissues in Immune Regulation"
Davos (CH) / 13-16.03.2013

Institute for Biological Research "Sinisa Stankovic", University of Belgrade
Belgrade (RS) / 23.07.2013

"From the bench to the clinic", European Academy of Dermatology and Venereology (EADV) meeting
Bellinzona (CH) / 28.11.2013

Keystone Symposium 'Emerging cytokine networks'
Vancouver (CA) / 17-22.01.2014

European Workshop "microRNAs and Immune System"
Zurich (CH) / 8-10.04.2014

MRC & Asthma UK Centre in Allergic Mechanisms of Asthma, King's College London
London (UK) / 23.09.2014

Swiss Multiple Sclerosis Society Researcher Meeting
Basel (CH) / 26.09.2014

"From the bench to the clinic", European Academy of Dermatology and Venereology (EADV) meeting
Bellinzona (CH) / 04.12.2014

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 working on human monocyte-derived dendritic cells. In 1997 she became member of the Basel Institute and since 2000 she is group 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 of human Th17 cells and the characterization of two distinct types of pathogen-specific Th17 cells that produce IFN-γ or IL-10. More recently, her lab demonstrated that T cells elicited by pathogens or vaccines comprise not only clones polarized toward a single fate, but also clones whose progeny have acquired multiple fates. Studies in the mouse model identified mechanisms that control lymphocyte migration in lymph nodes and in the central nervous and that sustain Tfh cell responses and phenotype. For her scientific achievements, she 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, member of EMBO in 2011, and President of the Swiss Society for Allergology and Immunology for the period 2013-2015.

Research Focus

We study the human system to address fundamental questions in the context of the immune response to different classes of antigens, such as microbial pathogens, commensals, allergens or self-antigens to gain insights into mechanisms that promote T cell differentiation, expansion and survival. These studies are performed in healthy individuals as well as selected cohorts of patients with infectious, inflammatory or neoplastic diseases, or immunodeficiency. Our original approach based on the ex vivo analysis and in vitro culture of T cells is currently complemented with new powerful technologies of single cell transcriptomics and epigenetic profiling of promoters and enhancers. Next generation sequencing methods are used to analyze the clonal composition of T cell subsets ex vivo and to monitor the dynamics of antigen-specific T cell clones, to gain insights into intraclonal T cell diversification and to address the basis of T cell crossreactivity and heterologous immunity. Our aim is to understand mechanisms of immune function and dysfunction and to translate basic findings to the medical setting.

Team

Group Leader: Federica Sallusto, PhD > federica.sallusto@irb.usi.ch

Current Members: Dominik Aschenbrenner, PhD student - Camilla Basso, PhD student - Antonino Cassotta, PhD student - Corinne De Gregorio, PhD student - Jérémie Goldstein, PhD - Daniela Latorre, PhD - Roberta Marzi, PhD - Federico Mele, PhD student - Sara Natali, PhD student - Samuele Notarbartolo, PhD - Luana Perlini, Technician - Silvia Preite, PhD student -Tomasz Wypych, PhD student.

Former Members: Simone Becattini, PhD student (09.2014) - Elena Brenna, Undergraduate student - Tess M. Brodie, PhD student (06.2014) - Gabor Gyülvészi, PhD (02.2013) - Daniela Impellizzieri, PhD student (02.2013) - Florian Wimmers, Undergraduate student (07.2013)

Deciphering the class and specificity of the T cell response to pathogens and vaccines

Simone Becattini, Daniela Latorre, Federico Mele, Corinne De Gregorio, Antonino Cassotta and Federica Sallusto

It is well known that human effector and memory T cells are heterogeneous in terms of migratory capacity and cytokine production. In contrast, the T cell response to pathogens has been considered to be polarized to particular subsets of effector T cells, with viruses and intracellular bacteria inducing Th1 cells, while fungi and extracellular bacteria inducing Th17 cells. Using cell sorting of Th1, Th2, and Th17 cells followed by CFSE labelling, antigenic stimulation and next generation TCR Vβ sequencing, we were able to demonstrate that memory T cells specific for pathogens, such as *C. albicans* and *M. tuberculosis*, or the tetanus toxoid vaccine, could be present in all subsets, albeit at different frequencies. Interestingly, several clonotypes were present in more than one subset and, in some cases, even in all subsets, while other clonotypes were restricted to one particular subset (Figure 1). By cloning antigen-specific T cells from memory subsets we were able to isolate several T cell clones from Th1, Th2, non-classical CCR6$^+$ Th1* and Th17 subsets, and show that they share the same TCR but display different transcription factors, cytokine production and chemokine receptor expression, characteristic of the subset from which they were isolated. Naïve T cells primed in vitro by pathogen could also give rise to multiple fates, even within the progeny of a single cell. These data reveal an unexpected degree of inter-clonal and intra-clonal T cell heterogeneity and suggest that polarized responses result from the preferential expansion of T cell clones within a specific subset.

*** Becattini S., et al.**
Science 2015, 347: 400-406.

This work was done in collaboration with Mathilde Foglierini, Blanca Fernandez, Davide Corti, and Antonio Lanzavecchia, IRB, and Sander Kelderman, Ton N. Schumacher, Netherlands Cancer Institute (NL).

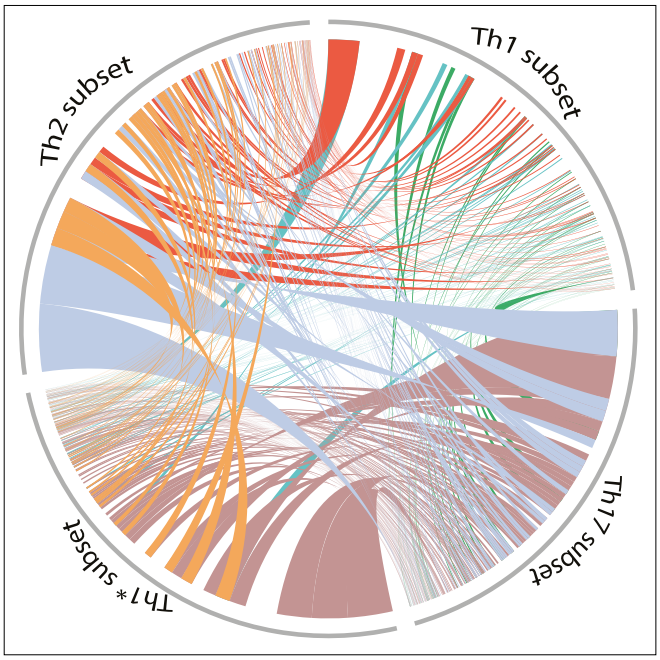


Figure 1
The sequences of the T cell receptor Vβ chains to determine the clonotype composition of human antigen-specific T cells in functionally distinct subsets can be obtained using the new methods of next generation DNA sequencing. This analysis showed that several clonotypes are present in more than one subset (connected by lines) and, in some cases, even in all subsets. Some highly expanded clones (represented by the thickest lines) are also shared between two or more subsets.

Analysis of the T cells against *C. albicans* in pathological conditions

Corinne De Gregorio, Simone Becattini, Federico Mele and Federica Sallusto

CD4$^+$ Th cells are crucial players in the adaptive immune response to pathogens. Th1 cells secrete IFN-γ and activate immunity to intracellular pathogens, Th2 cells secrete IL-4, IL-5 and IL-13 and are essential against extracellular parasites, while Th17 cells secrete IL-17A and IL-22 and protect from fungal pathogens such as *Candida albicans*. We developed a high throughput cell based assay that, combined with sorting of T cell subsets and with *in vitro* priming assays, can provide new insights on the class and specificity of the human T cell response to pathogens. With this approach we recently demonstrated that in healthy donors memory T cells specific for *C. albicans* are present at high frequency in CCR6$^+$ Th17 and Th1* subsets and at low frequency in CCR6$^-$ Th1 and Th2 subsets. Using next generation TCR Vβ sequencing, we also demonstrated that several clonotypes were present in more than one subset and, in some cases, in all subsets. We are now analyzing *C. albicans*-specific response in patients with Mendelian susceptibility to chronic mucocutaneous candidiasis (CMC), in order to evaluate the contribution of different T cell subsets to disease pathogenesis. Preliminary data show an altered distribution of *C. albicans*-specific T cells in memory subsets, with a high frequency of cells present in the CCR6$^-$ Th1 and Th2 subsets and a few present in the CCR6$^+$ Th17 and Th1* subsets. These studies are expected to improve our knowledge about disease pathogenesis and may be useful for the design of improved immunotherapeutic strategies.

This work is a joint project of the IRB, the Rockefeller University (US), and the Necker Medical School (FR) and is done together with Jean Laurent Casanova, Anne Puel, and Jacinta Bustamante.

Generation of multiple fates by priming of single human naïve CD4$^+$ T cells

Daniela Latorre and Federica Sallusto

We showed that memory CD4$^+$ T cells primed *in vivo* by pathogens or vaccines are highly heterogeneous, both at the population and at the clonal level. To test whether one round of stimulation could imprint heterogeneous fates within the progeny of naïve T cells, we primed *in vitro* a limited number of highly pure naïve CD4$^+$ T cells with *C. albicans* in the presence of autologous monocytes. Remarkably, proliferating CSFElo T cells recovered on day 15 produced IFN-γ, IL-17, IL-22 and IL-4, in various combinations. Using the cytokine secretion assay, we sorted IL-17$^+$ (IFN-γ$^-$, IL-4$^-$), IFN-γ$^+$ (IL-17$^-$, IL-4$^-$), and IL-4$^+$ (IL-17$^-$, IFN-γ$^-$) T cells, which were further expanded in bulk cultures and cloned by limiting dilution. Cytokine production by the bulk cultures was overall consistent with the cells being polarized. Among the 205 clones isolated and sequenced, 88 unique TCRβ sequences were identified, of which 21 were found in clones isolated from different polarized populations. Two expanded clonotypes were detected among cells that produced IL-17, IFN-γ or IL-4. These findings provided proof of concept that a single human naïve CD4 T cell can generate a heterogeneous progeny, even in a single round of antigenic stimulation. We have now set out an *in vitro* priming system in which a single antigen-specific naïve T cell is primed by a pathogen, and are planning single cell analysis to follow the fate of a single naïve T cell as it progresses through cell division and differentiation.

***Becattini S., et al.* ***
Science 2015, 347: 400-406.

Transcriptional analysis of human Th17 subsets and identification of a molecular signature for IFN-γ-secreting 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. In mice, TGF-β1 plus IL-6 induce Th17 cells that produce IL-10 and these Th17 cells are unable to induce experimental autoimmune encephalomyelitis (EAE). In humans, we have shown that IL-10-producing pathogen-specific Th17 cells are specifically induced in response to *Staphylococcus aureus*, whereas Th17 cells that coproduce IFN-γ are induced in response to *Candida albicans*. In this present study, we took advantage of analytical strategies in computational biology to integrate global gene expression data from diverse biological sources to investigate the degree to which human IFN-γ^+ vs. IFN-γ^- Th17 cells are similar to murine pathogenic vs. non-pathogenic Th17 cells using high throughput transcriptional analysis. Using a HuTh17 nanoString CodeSet that detects 419 genes, we found a marked similarity in the gene expression pattern between human IFN-γ^+ Th17 cells and mouse pathogenic Th17 cells as compared to human IFN-γ^- Th17 cells and mouse non-pathogenic Th17 cells, respectively. With all the transcriptomic data we have defined a pro-inflammatory molecular signature for human IFN-γ^+ Th17 cells that be utilized to better understand Th17 cells in the pathogenesis and treatment of human autoimmune diseases.

This work was a joint project between the IRB, the Harvard Medical School (US) and the Brigham and Women's Hospital (US) and was done together with Vijay K. Kuchroo, Howard L. Weiner, and Dan Hu.

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

Dominik Aschenbrenner, Samuele Notarbartolo and Federica Sallusto

In disease settings, two distinct types of Th17 cells have been characterized in mice and humans based on the range of effector cytokine production and the ability to induce disease. While autoreactive Th17 cells that were differentiated in the presence of IL-6, TGF-β and IL-23 were pathogenic in a mouse model of MS, Th17 cells differentiated in the presence of IL-6 and TGF-β were not. Pathogenic Th17 cells produced IL-17 together with IFN-γ, while non-pathogenic Th17 cells produced IL-17 and the immunoregulatory cytokine IL-10. In addition, each of these phenotypes was associated with a unique gene expression profile. We previously reported that in healthy donors, two types of memory Th17 cells can be identified that differ for the pattern of cytokines produced and the antigen recognized. *C. albicans*-specific Th17 cells co-produce IL-17 and IFN-γ, but not IL-10, while *S. aureus*-specific Th17 cells produce IL-17 without IFN-γ, but hold the ability to produce IL-10. We also showed that upon stimulation, most Th17 cells transiently downregulated their ability to produce IL-17, while a specific subset of Th17 cells, including *S. aureus*-reactive Th17 cells, reciprocally, upregulated production of IL-10. The downregulation of IL-17 was dependent on IL-2, which induced STAT5 phosphorylation that competed with STAT3 for binding to the *RORC* promoter, resulting in downregulation of RORγt transcription and expression, demonstrating a new function for IL-2 in regulating inflammatory Th17 responses (Figure 2). To investigate the transcriptional circuit that regulates expression of IL-10 in human Th17 cells we used a combination of transcriptional profiling and epigenetic approach. These studies demonstrated that c-MAF directly regulates *IL10* expression in human Th17 cells, but also revealed a more general role as positive or negative regulator of a sets of genes which are preferentially expressed in IL-10^{+} Th17 cells. These data suggest that c-MAF may represent a discriminative factor between pathogenic and non-pathogenic Th17 cells.

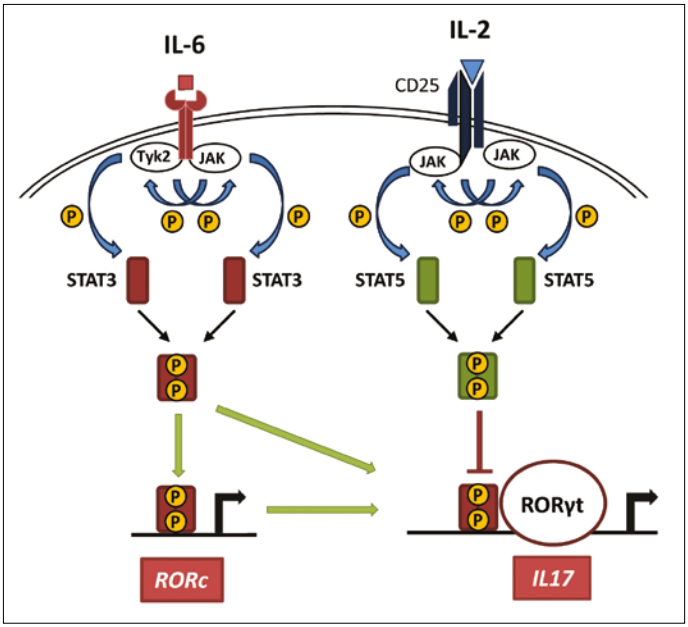


Figure 2
Model for the regulation of IL-17 production in human Th17 cells. High levels of pSTAT5 in activated Th17 cells would compete with pSTAT3 for binding to the IL17A locus and reduce IL-17 expression. Furthermore, pSTAT5 signaling may inhibit RORγt activity by direct interaction.

Pertussis toxin-driven IL-1β production by recruited myeloid cells is required for priming of encephalitogenic IFN-γ$^+$GM-CSF$^+$ Th17 cells

Camilla Basso, Francesca Ronchi*, Luana Perlini and Federica Sallusto*
**Equal contribution*

It is well established that IL-17-producing T cells (Th17) are heterogeneous in terms of cytokine production and capacity to initiate autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis. In this study we demonstrate that priming of pathogenic Th17 cells expressing RORγt and T-bet and producing IL-17A, IFN-γ and GM-CSF, but not IL-10, was dependent on the presence of pertussis toxin (PTX) at the time of immunization. PTX induced early production of IL-1β by CD11c$^+$ CCR2$^+$ Gr1$^+$ myeloid cells that were rapidly recruited to the antigen-draining lymph nodes after PTX injection. Recruitment of myeloid cells, induction of pathogenic Th17 cells, and development of EAE were impaired in IL-1β- and ASC-deficient mice, but not in mice lacking IL-12 and IL-23. Furthermore, PTX-induced production of IL-1β and generation of IFN-γ$^+$ GM-CSF$^+$ Th17 cells were impaired in mice depleted of Gr1$^+$ cells and in CCR2$^{-/-}$ mice, in which myeloid cells failed to migrate to lymph nodes but was recovered in mice receiving CCR2-sufficient inflammatory monocytes. The effect of PTX was independent of TLRs but required expression of MyD88 and IL-1R on both T and non-T cells. Collectively, these data shed light on the enigmatic function of PTX in the induction of EAE and reveal an in vivo mechanism for the generation of pathogenic Th17 cells.

Innate and adaptive immune response to *C. albicans*

Roberta Marzi, Gabor Gyülvészi, Simone Becattini, Luana Perlini and Federica Sallusto

Candida 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 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).

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

Federico Mele, Daniela Latorre and Federica Sallusto

Using the T cell library technique we recently showed that *Mycobacterium tuberculosis* (MTB)-responding T cells were highly enriched in libraries derived from the CCR6$^+$CXCR3$^+$ T cell subset (enriched in non-classis 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 several latent TB infected donors, with more than 80% of the MTB-reactive memory CD4 T cell response residing in the CXCR3$^+$CCR6$^+$ subset. TCR repertoire analysis of MTB-specific T cells in the different memory subsets by deep sequencing showed that there is little clonotype sharing between the MTB-reactive non-classic Th1* and Th17 cells, suggesting an independent origin. These studies are now continuing by comparing donors exposed to different antigens (BCG vaccine, MTB, environmental Mycobacteria), cells from blood or tissues (bronchoalveolar lavage) or before and after treatments.

***Lindestam Arlehamn C.S., et al.** **
PLoS Pathog 2013, 9:e1003130.

***Lindestam Arlehamn C.S., et al.** **
Proc Natl Acad Sci USA 2015, 112:E147-155.

This work is part of a project coordinated at La Jolla Institute for Allergy and Immunology (US), and is done in collaboration with Alessandro Sette, Bjoern Peters and Cecilia S. Lindestam Arlehamn.

Dissecting the CD4$^+$ T cell memory repertoire against Influenza virus

Antonino Cassotta and Federica Sallusto

Influenza viruses represent a major public health concern, due to their pandemic potential and to the sporadic spread of highly pathogenic strains, like H5N1, from zoonotic hosts to humans. The high mutation rate of Influenza viruses is the basis of their seasonal recurrence: one of the most immunogenic proteins of Influenza viruses is hemagglutinin, which is the main target of neutralizing antibodies. Protective immunity to Influenza viruses requires both antibody production by B cells and T cell responses: in particular the follicular helper CD4$^+$ T cell compartment has a prominent role in enhancing the B cell response. The surface expression of the chemokine receptor CXCR5 confer to T$_{FH}$ cells the capability to migrate in the germinal centers of lymphoid organs, where they contribute to class switch and somatic hypermutation of B cells, required for the production of high-affinity neutralizing antibodies. To dissect the memory CD4$^+$ T cell response after Influenza vaccination, we perform next generation sequencing of TCR-Vβ on circulating T$_{FH}$, T central memory (T$_{CM}$) and T effector memory (T$_{EM}$) cells isolated at multiple time points from the same blood donor. This allows us to decipher at clonal level the dynamics during time of the memory response to vaccination and the relationships between different T cell compartments. Moreover, by combining *in vitro* antigenic stimulation and T cell cloning, we systematically outline the dominance hierarchy of hemagglutinin-derived epitopes and the degree of crossreactivity with peptides derived from other influenza strains. Since we have previously reported that the antibody response to the HA stem is subdominant, we are interested to determine which might be the extrinsic factors that limit such a response. In particular we are performing experiment to test the hypotheses that the anti-stem response may be limited by the availability of T cell help or by the low abundance or instability of the relevant stem epitopes which are exclusively present in the pre-fusion HA. The efforts to understand the dynamics of CD4$^+$ T memory responses, the extent of heterosubtypic immunity to Influenza viruses, and a preferential parings between T and B cells specific for HA could have important implications for the design of new vaccination strategies, based also on the

exploitation of pre-existing crossreactive T cell compartments to enhance and direct neutralizing antibody responses against emerging highly pathogenic Influenza strains.

This work is being done in collaboration with Antonio Lanzavecchia, Kathrin Pieper, and Chiara Silacci, IRB.

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

Sara Natali, Tess M. Brodie, Elena Brenna and Federica Sallusto

Allergen-specific Th2 cells orchestrate allergic responses through cytokine and chemokine secretion, yet their phenotype remains unclear as multiple cytokines and chemokine receptors have been implicated in Th2 responses. In this study, we performed repertoire analysis on diverse T helper subsets identified using different combinations of chemokine receptors in allergic and non-allergic donors for both perennial and seasonal allergens. In allergic donors, the highest frequency of T cells reactive against house dust mite (HDM), Timothy grass (TG), European white birch (EWB), and Ragweed (RW) was found in a subset of memory T cells expressing the prostaglandin D2 receptor CRTh2, but responding T cells were also found in a $CCR4^+$ Th2 subset and, in the case of HDM, in a $CCR10^+$ T cell subset. $CRTh2^+$ T cells responded mostly to peptides from major allergens, had high functional avidity and correlated best with allergen specific IgE. These cells released IL-4, IL-5, IL-9, and IL-13 in response to all allergens, while $CCR10^+$ T cells produced primarily IL-22 in response to HDM. IL-9 production was flexible in $CRTh2^+$ cells and it was induced by strong co-stimulatory signals and TGF-β. Next generation sequencing is being performed to define the TCR repertoire of allergen-reactive T lymphocytes in the different memory subsets. In conclusion, our study revealed the heterogeneous and multifunctional phenotype of allergen-reactive T lymphocytes.

* ***Oseroff C., et. al.***
J. Immunol. 2012, 189:1800-1811.

* ***Brodie T., et al.***
Cytometry A 2013. doi: 10.1002/cyto.a.22278.

This work is being done in collaboration with Alessandro Sette and Cecilia S. Lindestam Arlehamn, La Jolla Institute for Allergy and Immunology (US).

Lung B cells promote ongoing allergic inflammation in a mouse model of asthma via B cell receptor-independent antigen presentation

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 importance of B cells to present antigens for antibody production is well documented. In contrast, very little is known about their capacity to influence T cell response in ongoing allergic inflammation. Using a mouse model of allergic asthma, we observed that lung B cells upregulated expression of MHC-II, CD86 and OX40L upon house dust mite (HDM) challenge and efficiently presented antigen to T cells *in vitro*. B cell depletion during challenge severely impaired expansion of activated T cells and their capacity to secrete Th2-type cytokines. Interestingly, efficient HDM presentation was a property not limited to B cells carrying surface Ig specific for HDM, since naïve, memory and B cells of unrelated specificities were equally efficient. Preliminary data suggest the existence of receptor-

mediated uptake in the process. Collectively, we highlight the existence of a novel mechanism that may lead to exacerbation of the allergic response.

This work is being done in collaboration with Laurent Perez, IRB.

Antibody affinity maturation by somatic hypermutation is impaired in the presence of high number of T follicular helper cells but restored by T regulatory cells

Silvia Preite, Camilla Basso, Francesca Ronchi, Dirk Baumjohann, Luana Perlini and Federica Sallusto*
**Former Lab member*

T follicular helper (Tfh) cells are required for the generation of high affinity antibody responses in germinal centers. Here, we studied the regulation of B cell responses by Tfh cells in immunized *Cd3e*-deficient mice reconstituted with small numbers of TCR transgenic $CD4^+$ T cells. Confirming an earlier report, in these mice a strong and sustained germinal center (GC) reaction was induced as a consequence of the very high numbers of Tfh cells that are generated compared to wild type (WT) mice. We now show, however, that the GCs generated in *Cd3e*-deficient mice had an altered distribution of B cells within the dark and light zones, which impaired the generation of antigen-specific high-affinity B cells and long-lived plasma cells. More specifically, while GC B cells in WT mice underwent affinity maturation through accumulation of somatic mutations in the framework/complementary-determining regions (FR/CDR) of the variable heavy (VH) chain gene, the GC B cells from *Cd3e*-deficient mice had reduced levels of activation induced deaminase (AID) and reduced numbers of somatic mutations. Strikingly, pre-reconstitution of *Cd3e*-deficient mice with regulatory T cells (Treg) was sufficient to restore GC B cell number and distribution in dark and light zones, rate of somatic mutations and generation of high affinity antibodies. Together with the previous study, these findings underline the importance of a quantitatively regulated Tfh cell response for somatic hypermutation, antibody affinity maturation and long lasting serological responses.

This work was done in collaboration with Antonio Lanzavecchia, Davide Corti, Mathilde Foglierini, Blanca Maria Fernandez Rodriguez, IRB.

The human immune T cell response to commensals.

Jérémie Goldstein and Federica Sallusto

It is now becoming increasingly appreciated that gut commensal microbiota plays an important role in shaping the immune system, particularly T cell differentiation. Dysbiosis of microbiota have been associated with numerous pathologies, making them interesting targets for new therapies. Our project focus on characterizing the repertoire and function of human gut commensal-specific T cells. We observed that commensal-specific $CD4^+$ T cells could be reproducibly detected in the blood of healthy donors, albeit in low frequency. These cells responded mostly to Gram-negative bacteria, such as *Escherichia coli* or *Enterobacter aerogenes*, but poorly to Gram-positive bacteria. Commensal-specific T cells were found mainly in a $CCR6^+$ Th17 subset, while they were rare in the T cell subsets expressing the gut-homing receptors CCR9 and/or $\alpha4\beta7$. Furthermore, reactivity to gut commensals was found in $Foxp3^+$ $CD4^+$ circulating Treg cells. Interestingly, T cell clones specific for commensal bacteria showed a high degree of cross-reactivity with other commensal bacteria but also pathogenic bacteria. However, commensal-specific clones did not cross-react with viral antigens. The nature and extent o cross-reactivity is currently being investigated.

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

The oral commensal *Streptococcus mitis* shows a mixed memory Th cell signature that is similar to and cross-reactive with *Streptococcus pneumoniae*.

Stian A. Engen, Simone Becattini, David Jarrossay and Federica Sallusto

Carriage of and infection with *Streptococcus pneumoniae* is known to predominantly induce Th17 responses in humans, but the types of Th cells showing reactivity towards commensal streptococci with low pathogenic potential, such as the oral commensals *S. mitis* and *S. salivarius*, remain uncharacterized. In this study we isolated memory $CD4^+$ T helper (Th) cell subsets from healthy human blood donors according to differential expression of chemokine receptors, expanded *in vitro* using polyclonal stimuli and characterized for reactivity against different streptococcal strains. We found that Th cells responding to *S. mitis*, *S. salivarius* and *S. pneumoniae* were predominantly in a $CCR6^+$ $CXCR3^+$ subset and produced IFN-γ, and in a $CCR6^+$ $CCR4^+$ subset and produced IL-17 and IL-22. Frequencies of *S. pneumoniae*-reactive Th cells were higher than frequencies of *S. mitis*- and *S. salivarius*-specific Th cells. *S. mitis* and *S. pneumoniae* isogenic capsule knockout mutants and a *S. mitis* mutant expressing the serotype 4 capsule of *S. pneumoniae* showed no different Th cell responses as compared to wild type strains. *S. mitis*-specific Th17 cells showed cross-reactivity with *S. pneumoniae*. As Th17 cells partly control clearance of *S. pneumoniae*, cross-reactive Th17 cells that may be induced by commensal bacterial species may influence the immune response, independent of capsule expression.

*** Engen, S. A., et al.** *PLoS One. 2014; 9:e104306.*

This work was a joint project between the IRB and the University of Oslo (NO) and was done together with Karl Schenck and Stian Engen.

The autoimmune basis of narcolepsy

Daniela Latorre, Eric Armentani and Federica Sallusto

Narcolepsy is a rare chronic neurological sleep-wake disorder that is caused by the selective loss of neuronal cells of the posterior hypothalamus that produce the neuropeptide hypocretin (HCRT). Accumulating lines of evidence support the notion that narcolepsy is an immune-mediated disorder that manifests in genetically predisposed individuals upon exposure to environmental factors. Despite numerous efforts, however, the definitive proof for autoimmunity, i.e. the presence of T cells or autoantibodies directed toward hypocretin cell antigens, has been lacking. Recently, a link between infection and narcolepsy was proposed based on cases of narcolepsy that follow *Streptococcus pyogenes* infection or H1N1 influenza infection or vaccination. By studying the specificity, function and antigen-receptor repertoire of T cells and other immune cells isolated from blood and cerospinal fluid of narcoleptic patients, our aims are to gain insights into basic pathogenetic mechanisms of narcolepsy and to define possible targets for immune intervention.

From the analysis of the human immune response to a Center of Medical Immunology

Federica Sallusto and Antonio Lanzavecchia

Our efforts towards human immunology as an example of personalized medicine have resulted in the development of high-throughput cell-based screening methods that can provide a wealth of information to address fundamental questions in immune-mediated mechanisms of host protection or disease. We have already shown that it is possible to integrate cellular and molecular platforms to dissect the human immune response to pathogens, commensals, neo-antigens and self-antigens. We have already established a cell bank of more than 1000 blood donors that can be interrogated to study specific immune responses to microbes and commensals. We have shown how the new technologies developed in our laboratory can be used to study mechanisms of disease and discover therapeutic antibodies and vaccines. We aim to further expand these efforts by improving and set up new technologies to dissect the extraordinary complexity of the human immune response and by reinforcing collaborations with clinical scientists in University Hospitals in Switzerland and abroad that have well characterized clinical cohorts of patients with immunodeficiency or with infectious, autoimmune, allergic or neoplastic diseases.

Funding

European Union
NEWTBVAC: Discovery and Pre-Clinical Development of New Generation Tuberculosis Vaccines
241745 / 2010 – 2013

Swiss National Science Foundation
Effector and memory T cell subsets in man and mouse
3100A0_131092 / 2010 – 2013

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

Swiss National Science Foundation
ProDoc Cell Migration
PDFMP3_137127 / 2011 – 2015

European Union
ABIRISK: Anti-Biopharmaceutical Immunization: Prediction and Analysis of Clinical Relevance to Minimize the Risk
IMI-2010- Call3- 115303 / 2012 – 2017

Swiss MS-Society
Investigating the cellular and molecular mechanisms involved in the migration of human CD4+ effector/memory T cell subsets across novel human in vitro models of the blood-brain barrier and the blood-cerebrospinal fluid barrier
2013

European Research Council (ERC)
Dissecting the human T cell response to pathogens, allergens, and self-antigens
ERC-2012-AdG-20120314-323183 / 2013 – 2018

Swiss National Science Foundation (SNF)
Studies on T cell activation, differentiation and plasticity in humans
310030_149475 / 2013 – 2016

Swiss National Science Foundation (SNF)
Sinergia UnmetMS: Unraveling the immune etiology of multiple sclerosis
CRSII3_154483 / 2014 – 2017

Collaborations

Burkhard Becher
University of Zurich (CH)

Jean Laurent Casanova
Rockefeller University, New York, NY (US)

Britta Engelhardt and Jens Stein
Theodor Kocher Institute, University of Bern (CH)

Marco Gattorno
Institute G. Gaslini, Genova (IT)

Cem Gabay
University Hospital, Geneva (CH)

Vijay K. Kuchroo
Harvard Medical School, Boston, MA (US)

Riitta Lahesma
Turku Centre for Biotechnology, University of Turku (FL)

Salomé LeibundGut
ETH Zurich (CH)

Dan Littman
New York University Medical School, New York, NY (US)

Andrew McMichael
University of Oxford, John Radcliffe Hospital, Oxford (UK)

Mihai G. Netea
Radboud University Nijmegen Medical Center, Nijmegen (NL)

Pawel Pelczar
Institute of Laboratory Animal Science, University of Zurich (CH)

Orlando Petrini
Microbiology Institute, Bellinzona (CH)

Eliane Piaggio
Institut Curie, Paris (FR)

Anne Puel
Imagine, Necker Hospital, Paris (FR)

Karl Schenck
University of Oslo (NO)

Ton N. Schumacher
Netherlands Cancer Institute, Amsterdam (NL)

Alessandro Sette
La Jolla Institute for Allergy and Immunology, La Jolla, CA (US)

Cameron Simmons
Oxford University, Ho Chi Minh City (VN)

Antonio Uccelli
University of Genova, Genova (IT)

Rudolf Valenta
Medical University of Vienna (AT)

Georgina Xanthou
Biomedical Research Foundation of the Academy of Athens, Athens (GR)

Gianna Zambruno
Istituto Dermatopatico dell'Immacolata, Roma (IT)

Visiting Scientists

Stian A. Engen
University of Oslo (NO)

Maria Cristina Gagliardi
Istituto Superiore di Sanità, Rome (IT)

Sofia Tousa
Biomedical Research Foundation of the Academy of Athens, Athens (GR)

Publications

ERK phosphorylation and miR-181a expression modulate activation of human memory Th17 cells.
Mele, F., C. Basso, C. Leoni, D. Aschenbrenner, S. Becattini, D. Latorre, A. Lanzavecchia, F. Sallusto and S. Monticelli
Nat Commun. 2015; 6:6431.

Immunological consequences of intragenus conservation of Mycobacterium tuberculosis T-cell epitopes.
Lindestam Arlehamn, C. S., S. Paul, F. Mele, C. Huang, J. A. Greenbaum, R. Vita, J. Sidney, B. Peters, F. Sallusto and A. Sette
Proc Natl Acad Sci U S A. 2015; 112:E147-155.

T cell immunity. Functional heterogeneity of human memory CD4(+) T cell clones primed by pathogens or vaccines.
Becattini, S., D. Latorre, F. Mele, M. Foglierini, C. De Gregorio, A. Cassotta, B. Fernandez, S. Kelderman, T. N. Schumacher, D. Corti, A. Lanzavecchia and F. Sallusto
Science. 2015; 347:400-406.

Antibody-driven design of a human cytomegalovirus gHgLpUL128L subunit vaccine that selectively elicits potent neutralizing antibodies.
Kabanova, A., L. Perez, D. Lilleri, J. Marcandalli, G. Agatic, S. Becattini, S. Preite, D. Fuschillo, E. Percivalle, F. Sallusto, G. Gerna, D. Corti and A. Lanzavecchia
Proc Natl Acad Sci U S A. 2014; 111:17965-17970.

FRT - Fondation Rene Touraine: Pour La Dermatologie.
Sakaguchi, S., F. Sallusto, T. Gebhardt, G. Stingl, R. A. Clark, L. F. Santamaria-Babi, E. Guttman-Yassky, G. Schuler and H. J. Koenen
Exp Dermatol. 2014; 23:772-785.

Rapid development of broadly influenza neutralizing antibodies through redundant mutations.
Pappas, L., M. Foglierini, L. Piccoli, N. L. Kallewaard, F. Turrini, C. Silacci, B. Fernandez-Rodriguez, G. Agatic, I. Giacchetto-Sasselli, G. Pellicciotta, F. Sallusto, Q. Zhu, E. Vicenzi, D. Corti and A. Lanzavecchia
Nature. 2014; 516:418-422.

The oral commensal Streptococcus mitis shows a mixed memory Th cell signature that is similar to and cross-reactive with Streptococcus pneumoniae.
Engen, S. A., H. Valen Rukke, S. Becattini, D. Jarrossay, I. J. Blix, F. C. Petersen, F. Sallusto and K. Schenck
PLoS One. 2014; 9:e104306.

Proteome-wide analysis of HIV-specific naive and memory CD4+ T cells in unexposed blood donors.
Campion, S. L., T. M. Brodie, W. Fischer, B. T. Korber, A. Rossetti, N. Goonetilleke, A. J. McMichael and F. Sallusto
J Exp Med. 2014; 211:1273-1280.

The many faces of CD4 T cells: Roles in immunity and disease.
Sallusto, F. and S. Monticelli
Semin Immunol. 2013; 25:249-251.

The who's who of T-cell differentiation: Human memory T-cell subsets.
Mahnke, Y. D., T. M. Brodie, F. Sallusto, M. Roederer and E. Lugli
Eur J Immunol. 2013; 43:2797-2809.

DCs: a dual bridge to protective immunity.
Sallusto, F.
Nat Immunol. 2013; 14:890-891.

Cross-neutralization of four paramyxoviruses by a human monoclonal antibody.
Corti, D., S. Bianchi, F. Vanzetta, A. Minola, L. Perez, G. Agatic, B. Guarino, C. Silacci, J. Marcandalli, B. J. Marsland, A. Piralla, E. Percivalle, F. Sallusto, F. Baldanti and A. Lanzavecchia
Nature. 2013; 501:439-443.

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; 121:4110-4114.

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
PLoS One. 2013; 8:e59863.

OMIP-018: Chemokine receptor expression on human T helper cells.
Brodie, T., E. Brenna and F. Sallusto
Cytometry A. 2013; 83:530-532.

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
Immunity. 2013; 38:596-605.

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.

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.

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.

Lectures and Seminars

Keystone Symposium "Advances in the Knowledge and Treatment of Autoimmunity"
Whistler, BC (CA) / 4-9.4.2013

46th Annual Canadian Society for Immunology Spring Meeting
Whistler, BC (CA) / 5-8.4.2013

University of Turku, Turku Centre for Biotechnology
Guest Seminar "Studying naïve and memory T cells in humans"
Turku (FL) / 25.4.2013

Università della Svizzera Italiana - Dies Academicus
Lecture "Alla scoperta del sistema immunitario"
Lugano (CH) / 4.5.2013

Cold Spring Harbor Symposia on Quantitative Biology "Immunity and Tolerance"
Cold Spring Harbor, NY (US) / 29.5-3.6.2013

23rd Regional Congress of the International Society of Blood Transfusion
Amsterdam (NL) / 2-5.6.2013

International Seminars in Virology
Bertinoro (IT) / 28-30.6.2013

15th International Congress of Immunology
Milan (IT) / 22-27.8.2013

XXXVI Annual Meeting of the Spanish Society for Biochemistry and Molecular Biology
Madrid (SP) / 4-6.9.2013

5th International Conference on Crossroads between Innate and Adaptive Immunity
Corfu (GR) / 21-26.9.2013

4th International Symposium on Regulators of Adaptive Immunity
Erlangen (DE) / 27-29.9.2013

11th International Cytokine and Interferon Society Meeting
San Francisco, CA (US) / 29.9-3.10.2013

Bern Immunology Club
Guest Seminar "Effector, regulatory and memory T cell subsets in humans"
Bern (CH) / 30.10.2013

EFIS-EJI Ruggero Ceppellini Advanced School of Immunology "Novel vaccination strategies against the three major killers"
Castellammare di Stabia (IT) / 16-20.10.2013

Interdisziplinäres Symposium "Inflammation and Immunology"
Munich (DE) / 20.11.2013

Cold Spring Harbor Laboratory Meeting "Harnessing Immunity to Prevent & Treat Disease"
Cold Spring Harbor, NY (US) / 20-23.11.2013

ETH-University Zurich MIM Graduate Program – 8th Introductory Course
Zurich (CH) / 13-17.1.2014

Keystone Symposium "Emerging Cytokine Network"
Vancouver (CA) / 17-22.1.2014

3rd NIF Winter School on Advanced Immunology
Awaji Island (JP) / 18-24.1.2014

CCHD-IAI Joint Symposium "Bridging the gap"
Vienna (AT) / 12-14.2.2014

22nd Annual Henry Kunkel Society Meeting
New York, NY (US) / 3-5.4.2014

2014 IgV Master Class
Lecture: Studies on human T cell response: differentiation, migration and function
Melbourne (AU) / 5.5.2014

5th Australasian Vaccine & Immunotherapeutics Development Meeting
Melbourne (AU) / 7-9.5.2014

EMBO Conference "Signaling in the Immune System"
Bertinoro (IT) / 17-21-5.2014

Gordon Conference "Immunochemistry and Immunobiology"
Newry, ME (US) / 1-6.6.2014

Riken IMS-JSI International Symposium on Immunology
Yokohama (JP) / 26-27.6.2014

FASEB Research Conference "Biology of the Immune System"
Snowmass, UT (US) / 29.6-4.7.2014

ETH Zurich
Guest Seminar: Dissecting the heterogeneity of human memory T cells primed by pathogens
Zurich (CH) / 19.8.2014

13th International Symposium on Dendritic Cells
Tours (FR) / 14-18.9.2014

44th Annual Congress German Society for Immunology
Bonn (DE) / 17-20.9.2014

3rd International Conference on Immune Tolerance
Amsterdam (NL) / 28-30.9.2014

XXXIX Meeting of the Brazilian Society of Immunology
Buzios (BR) / 18-22.10.2014

12th International Congress of Neuroimmunology
Mainz (DE) / 9-13.11.2014

University of Basel
Lecture Series in Advanced Immunology "Human T helper cells"
Basel (CH) / 14.11.2014

University of Basel
Guest Seminar "Class and Specificity of the Human T Cell Response in Health and Disease"
Basel (CH) / 14.11.2014

Fondation René Touraine Scientific Meeting "T cells and skin"
Paris (FR) / 5.12.2014

50th Annual Congress Dutch Society for Immunology
Kaatsheuvel (NL) / 17-19.12.2014

Midwinter Conference 2015
Seefeld (AT) / 17-21.1.2015

The Rockefeller University
Guest seminar "Dissecting the human T cell response in immunity, autoimmunity and allergy"
New York, NY (US) / 5.2.2015

Institut Pasteur "Journée de la Recherche Translationelle"
Paris (FR) / 13.2.2015

5th Milan Meets Immunology (MMI) meeting
Milano (IT) / 18.2.2015

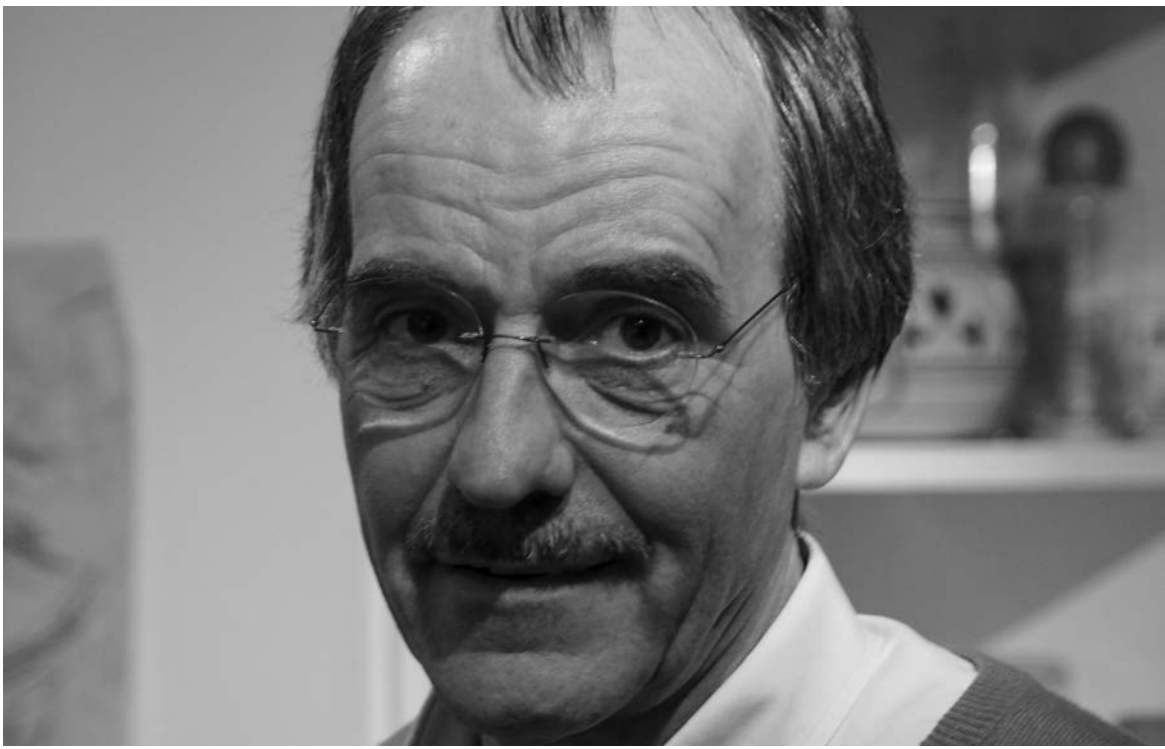
Keystone Symposium "T Cells: Regulation and Effector Function"
Snowbird, UT (US) / 29.3-3.4.2015

Cold Spring Harbor Laboratory Conference "Fundamental Immunology & Its Therapeutic Potential"
Cold Spring Harbor, NY (US) / 14-18.04.2015

The University of Chicago Medical Center
Guest seminar "Dissecting the human T cell response in immunity, autoimmunity and allergy"
Chicago, IL (US) / 23.4.2015

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 cytokine- 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

During development of mammalian organisms cells migrate along gradients to find their destinations. Orchestrated by the chemokine system, the most prominent cell movement is the continuous migration of immune cells engaged in host defense and immune surveillance. However, also non hematopoietic cells use the chemokine system for guidance, e.g. during neovascularization. It is therefore not surprising that some tumors adopted the migratory scheme to escape immune reactions. Locally produced chemokines form patterns on which cells can migrate through the activation of G-protein coupled chemokine receptors. An important aspect for the maintenance and local confinement of gradients is the requirement of sinks in apposition to the source of attractant. The atypical chemokine receptors (ACKR) were recently defined as a group of structurally related receptors for chemokines which mainly act as sinks and can promote cell migration through this activity. Since its discovery as receptor for CXCL12, ACKR3 (formerly CXCR7) emerged as critical regulator of the CXCR4/CXCL12 axis. ACKR3, which binds CXCL12 with higher affinity than CXCR4, modulates the activity of CXCR4 through the availability of CXCL12. Within the chemokine system CXCL12 and its signaling receptor CXCR4 distinguish by particular properties. Genetic deletion of either molecule leads to a comparable lethal phenotype. 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. 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 Gi-protein coupled receptor-mediated cell activation, ACKRs share the heptahelical structure of rhodopsin-like receptors, but do not couple to G-proteins. Despite the lack of signaling through G-proteins, ACKR3 may use biased signaling through arrestin. The receptor plays a critical role in development, as targeted deletion in mice is lethal. We have shown that ACKR3 in mammalian displays scavenger activity.

Team

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

Signaling of CXCR4 and ACKR3

Sabrina Casella, Sylvia Thelen and Marcus Thelen

G-protein coupled receptors (GPCRs) are known to display signaling bias depending on the ligand and the environment. Such signaling bias can be monitored by differential activation of pathways, such as downstream of G-proteins and arrestin. It is assumed that ligand-induced receptor active states represent conformations to which receptor associated proteins bind with different affinities. The interaction with different proteins depending on the signaling conditions is expected to be the starting point of signal bias. The project aims in the identification of novel receptor-protein interactions and to characterize receptor proteomes under different stimulatory conditions. To this end we fused the engineered ascorbate peroxidase APEX2 to the C-terminus of CXCR4 and ACKR3. Upon addition of hydrogen peroxide the enzyme biotin phenol into a radical which leads to the biotinylation of proteins in close proximity. A method is developed to recover biotinylated proteins from whole cell lysates for identification by mass spectrometry. The project is ongoing.

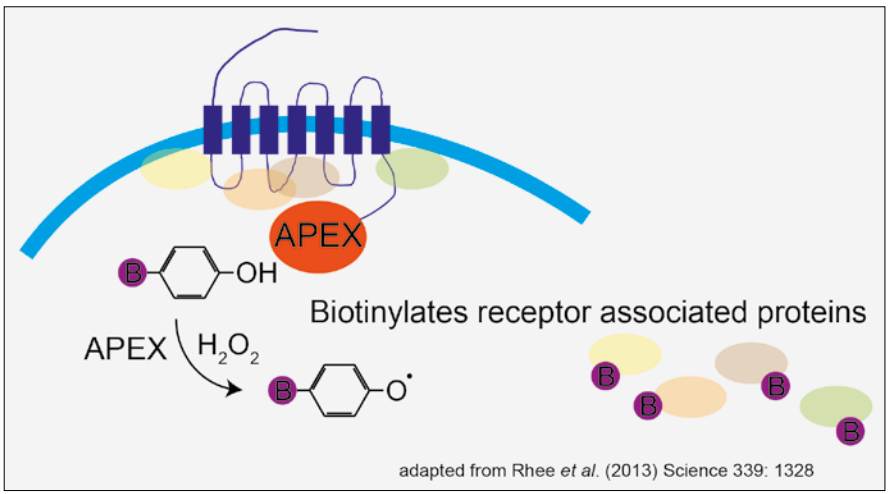


Figure 1
Characterization of receptor proteomes.
APEX2 tagged receptors are expressed in model cell lines. Proteins in the vicinity of the receptor under resting or stimulated conditions will be biotinylated through phenol radicals generated by the APEX2 enzyme in the presence of H_2O_2.

Molecular mechanisms of ACKR3 sorting and potential signaling properties

Marie-Luise Humpert and Marcus Thelen

– *Functional role of ACKR3 in B cells*. In the past we confirmed the expression and function of ACKR3 on primary human and mouse B cells. Complementary methods including mass spectrometry of immunoprecipitates were used to provide unequivocal evidence of the expression of ACKR3 on the cells. The functional role of ACKR3 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 ACKR3^{+} cells in human tonsils, one being memory B cells the other being plasmablasts. Moreover, we found an inverse correlation of ACKR3 and CXCR5 cell surface levels. While CXCR5 is present on follicular and early GC B cells, ACKR3 becomes transiently upregulated at the plasmablast stage. The findings suggest an important role of ACKR3 in regulating the migration at late stages of B cell maturation. The differential expression pattern on B cells is consistent with the hypothesis that plasmablasts loose responsiveness to CXCL12 and are therefore not retained by the chemokine in germinal centers. In fact, we showed that plasmablast migrate more efficiently towards CXCL12 when ACKR3 is attenuated by a specific monoclonal antibody.

***Humpert, M. L., et al.** **
Proteomics. 2012, 12:1938-1948.

***Humpert, M. L., et al.** **
Eur. J. Immunol: 2014, 44:694-705.

– *Investigations of ligand-dependent and -independent receptor trafficking to elucidate the mechanism of chemokine scavenging*. Intracellular trafficking of ACKR3 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. Moreover when epitope tagged CXCR4 and ACKR3 were contemporaneously expressed on HeLa cell and labeled with different fluorescent dyes both receptors colocalize at 17°C. However, shifting to 37°C, which allows endocytosis of plasma membrane proteins, only ACKR3 spontaneously and quantitatively internalizes leaving CXCR4 behind indicating that the two receptors do not from stable heterodimers.

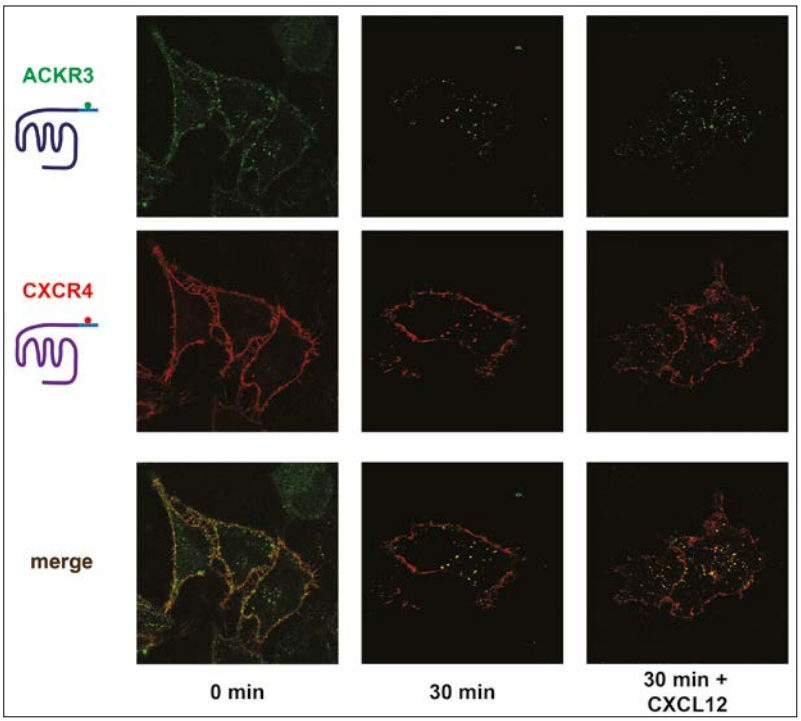


Figure 2
CXCR4 and ACKR3 endocytose independently.
Epitope tagged ACKR3 (upper panels) and CXCR4 (middle panels) were labeled at 17°C with Atto-488 and Atto-647-conjugated coenzyme A, respectively using ACP and PCP phosphopantetheinyl transferases on the surface of HeLa cells. Cells were fixed immediately (left column), shifted to 37°C for 30 min and fixed (middle columns) or stimulated for 30 min with CXCL12 (right columns). Images were taken with a confocal laser scan microscope (Leica SP5). ACKR3 largely internalizes spontaneously while CXCR4 requires the presence of CXCL12 for internalization.

Role of ACKR3 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 discovery of ACKR3 as atypical chemokine receptor for CXCL12, numerous studies investigated the expression of the receptor on tumor cells. The reports focus mostly on tumors of mesenchymal cell origin. We have shown that ACKR3 is expressed in B cells, in particular on plasmablasts of GCs. The high expression of ACKR3 during GC B cell differentiation correlates with the stage when cells have down regulated CXCR5, continue to express CXCR4 and 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 ACKR3 DLBCL and Burkitt's lymphoma with respect to its effects on modulating CXCR4-dependent responses.
A marked expression of ACKR3 is also observed in human skin B cell lymphomas. A prominent expression is seen on so called "immunocytes" which could represent plasmablast-like cells.

In vivo studies of ACKR3 function

Egle Radice and Marcus Thelen

We recently described ACKR3$^+$ plasmablasts of human tonsil B cells. Our findings are consistent with these cells being early, not fully differentiated plasma cells. Moreover, the data suggest that attenuation of ACKR3 activity in these cells negatively affects the egress of the cells from GCs and thereby the homing to bone marrow where the cells become long living antibody secreting plasma cells. To genetically confirm the necessity of ACKR3 expression at the plasmablast stage for efficient humoral protection of the host we are breeding mice with conditional deletion of ACKR3 with mice expressing the Cre-recombinase at different stages of B cell development. The lack of ACKR3 in the B cell compartment is investigated with respect to the ability of the animals to mount an efficient immune response to different pathogens. Targeted B cells express also 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.

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

Jost Reinhold Foundation
2010-2013

Collaborations

Antonio Alcami
Centro de Biología Molecular Severo Ochoa (CSIC), University of Madrid (ES)

Helmut Beltraminelli
Inselspital Bern (CH)

Bruno Fuchs
Klinik Balgrist (CH),

Peter Gierschick
University of Ulm (DE)

Nathan Karin
Technion, Haifa (IL)

Massimo Locati
University of Milan School of Medicine and Humanitas Clinical and Research Center, Milan (IT)

Silvano Sozzani
University of Brescia (IT)

Rolf Stahl
University Hospital Hamburg (DE)

Erez Raz
University of Münster (DE)

Antal Rot
University of York (UK)

Publications

Herpes simplex virus enhances chemokine function through modulation of receptor trafficking and oligomerization.
Martinez-Martin, N., A. Viejo-Borbolla, R. Martin, S. Blanco, J. L. Benovic, M. Thelen and A. Alcami
Nat Commun. 2015; 6:6163.

ATP-Gated Ionotropic P2X7 Receptor Controls Follicular T Helper Cell Numbers in Peyer's Patches to Promote Host-Microbiota Mutualism.
Proietti, M., V. Cornacchione, T. Rezzonico Jost, A. Romagnani, C. E. Faliti, L. Perruzza, R. Rigoni, E. Radaelli, F. Caprioli, S. Preziuso, B. Brannetti, M. Thelen, K. D. McCoy, E. Slack, E. Traggiai and F. Grassi
Immunity. 2014; 41:789-801.

CXCR7 prevents excessive CXCL12-mediated downregulation of CXCR4 in migrating cortical interneurons.
Abe, P., W. Mueller, D. Schutz, F. Mackay, M. Thelen, P. Zhang and R. Stumm
Development. 2014; 141:1857-1863.

CXCL11-dependent induction of FOXP3-negative regulatory T cells suppresses autoimmune encephalomyelitis.
Zohar, Y., G. Wildbaum, R. Novak, A. L. Salzman, M. Thelen, R. Alon, Y. Barsheshet, C. L. Karp and N. Karin
J Clin Invest. 2014; 124:2009-2022.

New nomenclature for atypical chemokine receptors.
Bachelerie, F., G. J. Graham, M. Locati, A. Mantovani, P. M. Murphy, R. Nibbs, A. Rot, S. Sozzani and M. Thelen
Nat Immunol. 2014; 15:207-208.

CXCR4 antibody treatment suppresses metastatic spread to the lung of intratibial human osteosarcoma xenografts in mice.
Brennecke, P., M. J. Arlt, C. Campanile, K. Husmann, A. Gvozdenovic, T. Apuzzo, M. Thelen, W. Born and B. Fuchs
Clin Exp Metastasis. 2014; 31:339-349.

CXCR7 influences the migration of B cells during maturation.
Humpert, M. L., D. Pinto, D. Jarrossay and M. Thelen
Eur J Immunol. 2014; 44:694-705.

International Union of Pharmacology. LXXXIX. Update on the Extended Family of Chemokine Receptors and Introducing a New Nomenclature for Atypical Chemokine Receptors.
Bachelerie, F., A. Ben-Baruch, A. M. Burkhardt, C. Combadiere, J. M. Farber, G. J. Graham, R. Horuk, A. H. Sparre-Ulrich, M. Locati, A. D. Luster, A. Mantovani, K. Matsushima, P. M. Murphy, R. Nibbs, H. Nomiyama, C. A. Power, A. E. Proudfoot, M. M. Rosenkilde, A. Rot, S. Sozzani, M. Thelen, O. Yoshie and A. Zlotnik
Pharmacol Rev. 2014; 66:1-79.

Expression of the Chemokine Receptor CXCR7 in CXCR4-Expressing Human 143B Osteosarcoma Cells Enhances Lung Metastasis of Intratibial Xenografts in SCID Mice.
Brennecke, P., M. J. Arlt, R. Muff, C. Campanile, A. Gvozdenovic, K. Husmann, N. Holzwarth, E. Cameroni, F. Ehrensperger, M. Thelen, W. Born and B. Fuchs
PLoS One. 2013; 8:e74045.

Immune response: steroids drive dendritic cells.
Jarrossay, D. and M. Thelen
Nat Immunol. 2013; 14:424-426.

Lectures and Seminars

University of Ghent
Why B cells (not) respond to CXCL12?
VIB Ghent (BE) / 17.04.2013

University of Iowa
Why B do cells (not) respond to CXCL12?
Immunology, Iowa City (US) / 23.09.2013

University of Brescia
Why B do cells (not) respond to CXCL12?
Brescia (IT) / 31.10.2013

University of Torino
Why B cells do (not) respond to CXCL12?
Torino (IT) / 18.11.2013

Actelion Pharmaceuticals
Cell migration governed by the atypical chemokine receptor ACKR3
Allschwil (CH) / 10.01.2014

Cytomeet University of Bern
Cell migration mediated by atypical chemokine receptors
Bern (CH) /14.01.2014

Gordon Research Conference, Chemotactic Cytokines
Regulation of Chemokine Function/Discussion leader – Introduction
West Dover (US) / 27.07.-1.08.2014

Vanderbilt University
CXCR7 (ACKR3) in B cells
Nashville (US) / 04.08.2014

Corte Franca Summit
CXCR7 (ACKR3) in B cells
Corte Franca (BS) / 25.-27.09.2014

University of Padova
The Chemokine System in Cancer
Padova (IT) / 21.11.2014

European Academy of Dermatology and Venerology
The chemokine decoy receptor ACKR3 in health and disease
Bellinzona (CH) / 04.12.2014

Mariagrazia Uguccioni

Chemokines in Immunity

Mariagrazia Uguccioni

Mariagrazia Uguccioni received a degree in Medicine from the University of Bologna (IT) where she specialized in Haematology in 1994. From 1993 to 2000 she was a member of the Theodor Kocher Institute, University of Bern (CH), and since 2000 she is group leader at the IRB, 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 continuing dissecting the mechanisms leading to chemokine synergism in leukocytes.

Research Focus

Our research interest remains focused on cell trafficking in physiology and pathology, with an emphasis on the mechanisms governing fine-tuning modulation of chemokine 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. Directional guidance of cells via gradients of chemokines is considered crucial, but we often lack in many pathological conditions, a direct evidence of chemokine receptor functionality, which may be relevant in the development of the disease, and can be modulated by the therapy. During the inflammatory response, from the onset to the chronic phase and even in the case of autoimmune diseases, the sequential release of exogenous agents (*e.g.*: bacterial and viral products) and induction of endogenous mediators (*e.g.*: cytokines, chemokines and DAMPS) contributes to the recruitment of circulating leukocytes to the inflamed site. There are many different ways to enhance or reduce the inflammatory response and to fine tune leukocytes recruitment. We have described a novel regulatory mechanism of leukocyte migration that shows how several non-ligand chemokines may trigger leukocytes to respond to agonist concentrations that *per se* would be inactive, thus lowering their "migratory threshold" ability. However, very little is known about the capacity of non-ligand molecules, other than chemokines, to synergize with chemokine agonists. Our studies are now focusing on deciphering the role of known chemokine heterocomplexes which act in synergism in inflammatory conditions, and also of the reciprocal interactions (synergism or antagonism) of cytokines and inflammation derived proteins, such as HMGB1.

Team

Group Leader: Mariagrazia Uguccioni, MD > mariagrazia.uguccioni@irb.usi.ch
Members: Michelle Berni, Master student – Valentina Cecchinato, PhD – Gianluca D'Agostino, PhD student – Maria Gabriela Danelon, Technician – Alessia Landi, Ph student – Michele Proietti, MD – Lorenzo Raeli, PhD

Chemokines: Structure/Function

Lorenzo Raeli, Gianluca D'Agostino, Gabriela Danelon and Mariagrazia Uguccioni

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

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 in collaboration with the groups of Marcus Thelen and Luca Varani at the IRB, of Marco Bianchi at the San Raffaele Institute (Milan, IT), and of Mario Mellado CSCI (Madrid, ES) (Figure 1).

We are now studying, in collaboration with the group of Costantino Pitzalis at the William Harvey Institute (London, UK) and of Antonio Manzo at the University of Pavia (Italy) the molecules which cooperate in cell recruitment and activation at inflammatory sites which are crucial in Rheumatoid Arthritis and might be modulated by the anti-cytokine therapy. This study may shed new light on the mechanisms which significantly "push back" inflammation by not only directly modulating cytokines activity, such as TNF, but also governing molecules that act in synergism with chemokines and that can be additional targets for novel anti-inflammatory strategies.

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

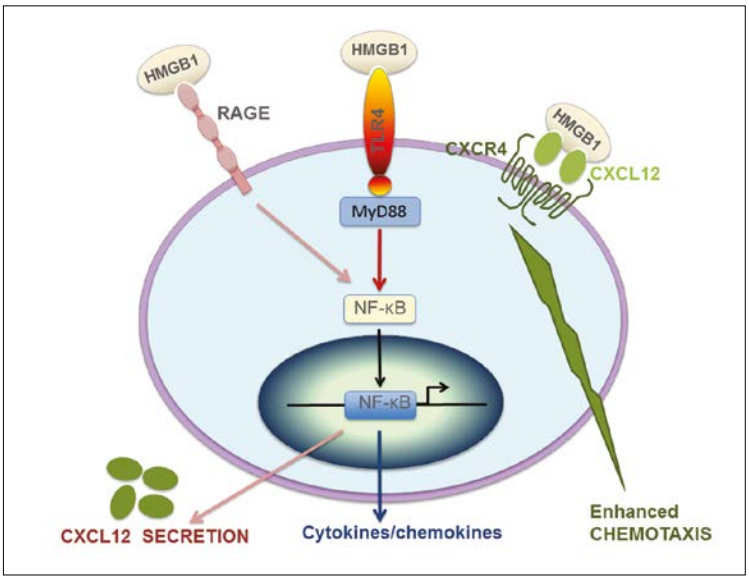


Figure 1
Model of action of HMGB1/CXCL12 synergism in leukocytes response.
Model of action of HMGB1/CXCL12 synergism in leukocytes response. HMGB1 binds CXCL12 and the heterocomplex activates CXCR4 and induces enhanced cell migration. HMGB1 binding to TLR4 and RAGE leads to the activation of NF-κB and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

Responses to chemokines in HIV/SIV infection

Valentina Cecchinato, Michele Proietti, Lorenzo Raeli, 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 Dr. Cecchinato in the mucosa of SIV infected animals. Nevertheless, the migratory capacity of this T cell subpopulation has not been investigated so far.

Chemokines are important mediators of leukocyte trafficking and function, and deregulation of their expression might contribute in part to the pathogenesis of HIV-1/SIV infection. In the frame of 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).

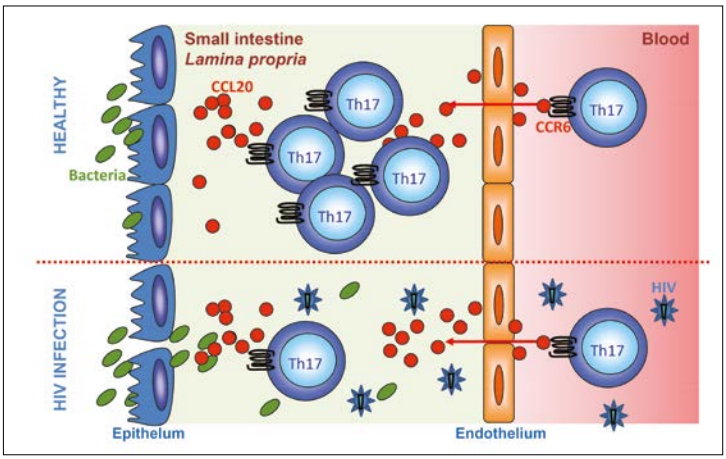


Figure 2
HIV reduces Th17 cells at mucosal sites.
We study the impact of chemokines and chemokine receptor expression on the migratory capacity of CCR6+ cells.

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

Alessia Landi, Gianluca D'Agostino 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/UL128-131A 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. 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, to assess its capability of modulating chemokine-induced responses.

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

Funding

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

European Union
TIMER: Targeting Novel Mechanisms of Resolution in Inflammation
FP7 –281608 / 2012-2015

Swiss National Science Foundation
Impact of multiple chemokine expression in human disease
310003A- 143718 / 2013-2016

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

Swiss National Science Foundation – Pro-Doc
Immune cell migration in immunosurveillance and inflammation - Soluble Factors in Cell Migration
141773 / 2012-2015

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

Gottfried und Julia Bangerter-Rhyner Foundation
Dampening Inflammation in Autoimmunity by Targeting Chemokine synergy-inducing molecules
2014

Collaborations

Marco Bianchi
San Raffaele Institute, Milan (IT)

Curzio Ruegg
University of Fribourg (CH)

Carole Bourquin
University of Fribourg (CH)

Marlene Wolf
Theodor Kocher Institute, University of Bern (CH)

Mario Mellado
Centro Nacional de Biotecnología, Madrid (ES)

Costantino Pitzalis
William Harvey Institute, London (UK)

Antonio Manzo
University of Pavia (IT)

Nikhil Yawalkar
Dermatology Department, University of Bern (CH)

Enos Bernasconi
Division of Infectious Diseases, Regional Hospital of Lugano (CH)

Roberto F. Speck
Division of Infectious Diseases and Hospital Epidemiology, University Hospital of Zurich (CH)

Christiane Stahl-Hennig
German Primate Center, Göttingen (DE)

Publications

Innate immune cells express IL-17A/F in acute generalized exanthematous pustulosis and generalized pustular psoriasis.
Kakeda, M., C. Schlapbach, G. Danelon, M. M. Tang, V. Cecchinato, N. Yawalkar and M. Uguccioni
Arch Dermatol Res. 2014; 306:933-938.

HMGB1 and leukocyte migration during trauma and sterile inflammation.
Venereau, E., M. Schiraldi, M. Uguccioni and M. E. Bianchi
Mol Immunol. 2013; 55:76-82.

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.

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

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.

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.

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

University of Verona
Synergy-inducing chemokines: a new model for chemokine interactions
Verona (IT) / 06.06.2013

15th International Congress of Immunology (ICI)
Synergy in the chemokine system: a new model for tuning chemokine activities
Milan (IT) / 25.08.2013

SIICA
CCR6+ T-cell Inefficient Actin Polymerization Prevents Mucosal Reconstitution in Long-Term Antiretroviral Therapy treated HIV-positive subjects
Firenze (IT) / 29.05.2014

Luca Varani
Structural Biology


COMPARATIVE ANATOMY AND HISTOLOGY
A MOUSE AND HUMAN ATLAS

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 Fellowship" in 2003. At Stanford he completed the first NMR study on TCR-pMHC complexes, proposing a novel approach to the systematic characterization of protein-protein interactions.
In October 2007, he joined the Institute for Research in Biomedicine (Bellinzona, CH) as a group leader in Structural Biology. In a highly multidisciplinary approach, he uses experimental information ranging from NMR to cellular assays to guide and validate computational simulations of antibody-pathogen interactions.

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. Computers can also simulate the natural movement of proteins other time (Molecular Dynamics). Computational models, 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, computational docking and molecular dynamics in single workflow, and to apply it to biologically relevant cases such as the interactions between antibodies and pathogens (infectious diseases and cancer) or between chemokines. We are also able to rationally modify proteins and antibodies with the purpose of improving their properties or test the role of particular residues in biochemical processes.

Team

Group Leader: Luca Varani, PhD > luca.varani@irb.usi.ch
Members: 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 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 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 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 engineering new antibodies with improved properties.

Dengue Virus

Dengue Virus (DENV) is 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: 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 could 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 and has the most variability through serotypes. Furthermore, DIII is the target of many neutralizing antibodies and it is relatively small, making it ideal for NMR and computational studies.

Our aim is to compare several 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.

On one hand we want to determine where and how antibodies bind, on the other hand we want to investigate the role of antigen flexibility and how it is affected by antibody binding (collaboration with Valente, Federal University of Rio de Janeiro).

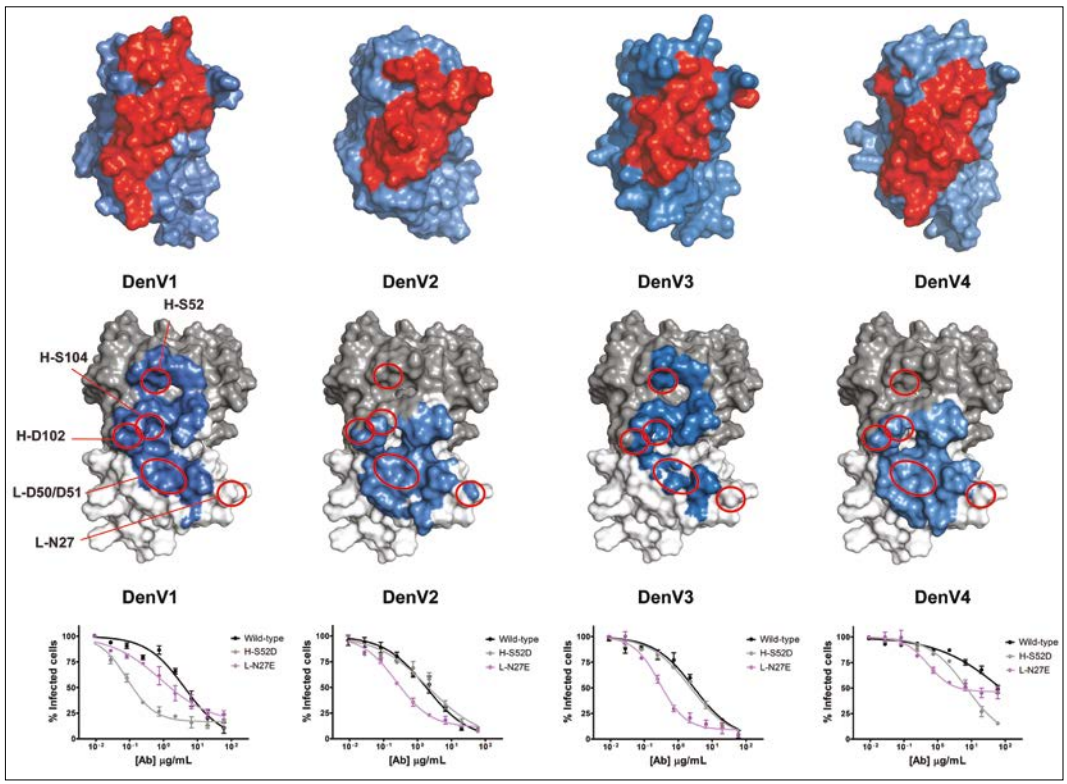


Figure 1

Our NMR validated computational docking approach allowed us to define the interface between antibody DV32.6 and DIII of Dengue virus, leading to the rational design of antibody mutants that neutralize the virus up to 40 times better than the original molecule. Top: NMR mapping of antibody DV32.6 on DIII; the surface of the antigen is shown in blue with epitope residues in red. Middle: antigen binding interface of antibody DV32.6, as defined by computational docking; residues interacting with the antigen are shown in blue; circles indicate selected residues that were mutated to rationally alter the antibody properties. Bottom: virus neutralization assays for DV32.6 wild-type (black) and two mutants (grey and violet); a shift to the left of the sigmoidal indicates increased neutralization, in comparison to the wild-type.

Rational antibody engineering to better neutralize Dengue

We recently demonstrated that our combined NMR/docking approach is sufficiently accurate to allow the rational design of antibody mutants 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 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. This was the first example of rational antibody design without the aid of crystallographic structures, to our knowledge, and proves that experimentally validated computational docking is an accurate, rapid and powerful tool for the characterization and rational engineering of antibodies.

Structural Characterization of a potent neutralizer of Dengue Virus

DV87 is possibly the most potent Dengue antibody described so far in the literature. It binds the surface protein of Dengue virus with nanomolar affinity and potently neutralizes the virus (IC50 0.004µg/ml). All anti-DIII antibodies with known structure bind to epitopes only partially accessible on the viral surface; this is the supposed cause of their limited ability to neutralize the virus. Antibody DV87 is a much stronger neutralizer. It is reasonable to expect that, in contrast to the previous antibodies, it binds to an accessible epitope. Surprisingly, we have shown this not to be the case; on the opposite, its novel epitope is even less accessible to the antibody. The hypothesis is that antibody binding requires a conformational change that disrupts the viral structure and prevents it from infecting the cells.
This work was done in collaboration with Antonio Lanzavecchia, IRB and Ana Paula Valente, University of Rio di Janeiro (BR).

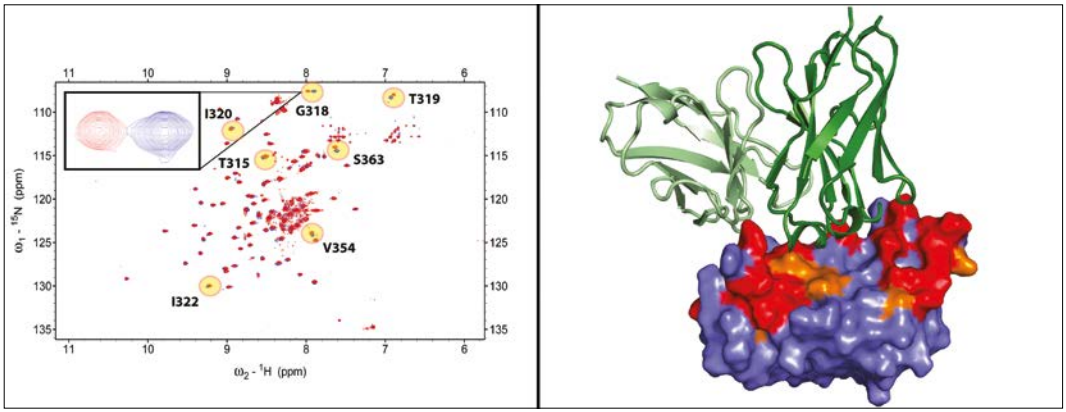


Figure 2
NMR epitope mapping (left) and computational model (right) of the complex between antibody DV87 (green cartoon) and DIII of Dengue virus (blue surface, epitope residues are shown in red and orange).

Antibodies as a tool to investigate prion protein toxicity

Marco Bardelli, Luca Simonelli and Luca Varani

Prion diseases are fatal neurodegenerative disorders affecting humans and animals for which no cure is available. Cellular prion protein, the causative agent, can convert into a toxic form (scrapie) capable of propagating to other prion molecules and ultimately leading to the accumulation of aggregates of prion protein in the brain through a largely unknown process. Aguzzi and co-workers have recently shown that some antibodies against the globular domain of prion protein can increase its toxicity. This has implication for therapy, since antibody treatment is considered a valid strategy, but also offers us a tool to investigate the toxic process.
A combination of computational and experimental techniques allowed us to ascertain the structural determinants for binding of antibody POM1 to prion protein, leading to increased toxicity. We are trying to discover the atomic-level events that, upon antibody binding, trigger the activation of prion protein toxicity. One hypothesis is that POM1 alters the conformational freedom of the prion, favouring states that are more prone to aggregation.
We generated antibody mutants that can still bind to prion protein but might have different toxicity. We are particularly interested in the effect of antibody binding on prion flexibility.
This work was done in collaboration with Adriano Aguzzi, University Hospital Zurich (CH).

	K_a(1/nMs)	K_d(1/s)	K_D(nM)	
POM1 wt	4.78×10^{4}	3.16×10^{-4}	5.08	
POM2 wt	5.33×10^{4}	3.62×10^{-3}	233	
POM1 W33A heavy	no binding	no binding	no binding	CDR_H1 loop
POM1 D52A heavy	3.34×10^{5}	4.56×10^{-2}	103	CDR_H2 loop
POM1 D55A heavy	1.05×10^{5}	3.67×10^{-2}	372	
POM1 Y57A heavy	7.7×10^{4}	3.15×10^{-2}	406	
POM1 Y101A heavy	6.26×10^{4}	7.14×10^{-4}	11.5	CDR_H2 loop
POM1 Y104A heavy	8.21×10^{3}	1.48×10^{-2}	2050	
POM1 S32A light	6.36×10^{4}	5.95×10^{-4}	9.98	CDR_L1 loop
POM1 Y50A light	5.7×10^{4}	8.17×10^{-3}	313	CDR_L2 loop
POM1 S91A light	1.34×10^{4}	1.85×10^{-2}	1430	CDR_L3 loop
POM1 W94A light	3.08×10^{5}	2.7×10^{-2}	201	
POM1 Y96A light	6.24×10^{4}	7.4×10^{-3}	123	

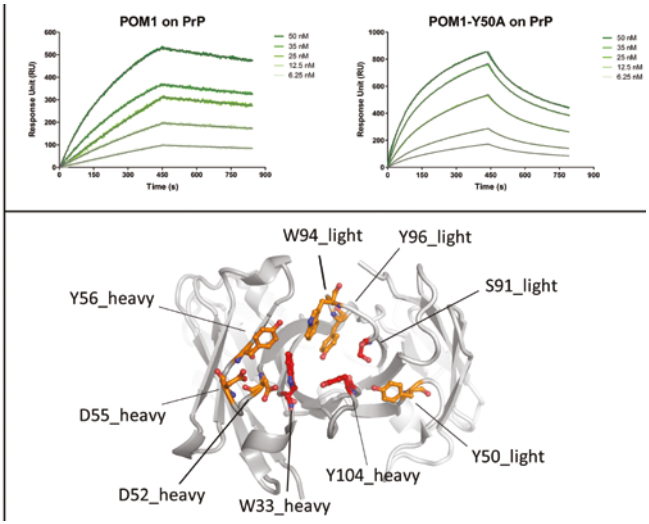


Figure 3

Characterization of the structural determinants for binding of POM1 antibody to prion protein. Computational simulations and visual structural analysis suggested antibody residues thought to be important for binding. Mutating them had an impact on antibody affinity (SPR traces and table on the left; mutations impaired binding to a greater extent going from yellow to orange and red). Critical residues form a relatively flat, circular interface in the middle of the antigen binding site and they are shown as colored stick in the cartoon representation of the antibody structure (gray).

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 where vaccination programs are not fully enforced. Immigration of non-immunized individuals from affected countries is a concern due to the fact that the vaccine does not confer long lasting protection. In the 1990s, for instance, an epidemic caused approximately four thousand deaths in Russia even amongst formerly vaccinated individual. Besides 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 which are very potent neutralizers. We have characterized their biophysical and biochemical properties with the intent of elucidating the determinants for efficient toxin neutralization. It is not uncommon to believe that antibody binding to any site of a relatively small protein such as DT is sufficient for toxin neutralization, which would thus mainly depend on the antibody binding properties (on-rate, off-rate and overall affinity). By contrast, neutralization of large molecular entities such as viruses usually requires targeting of specific sites. We have shown that, unexpectedly, there is no direct correlation between binding and neutralization. The most efficient antibodies of our panel bind to the receptor binding domain of DT. It is easy to expect that they can, thus, neutralize DT by inhibiting the interaction with its cellular receptor. Surprisingly, this is not the case. The best neutralizers appear to inhibit the pH dependent conformational change required for DT to interact with lipid membranes. Our hypothesis, that we are trying to verify with confocal microscopy and other cellular assays, is that the most potent antibodies in our panel prevent the translocation of DT catalytic domain through the endosomal membrane, a key step for DT toxicity.

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

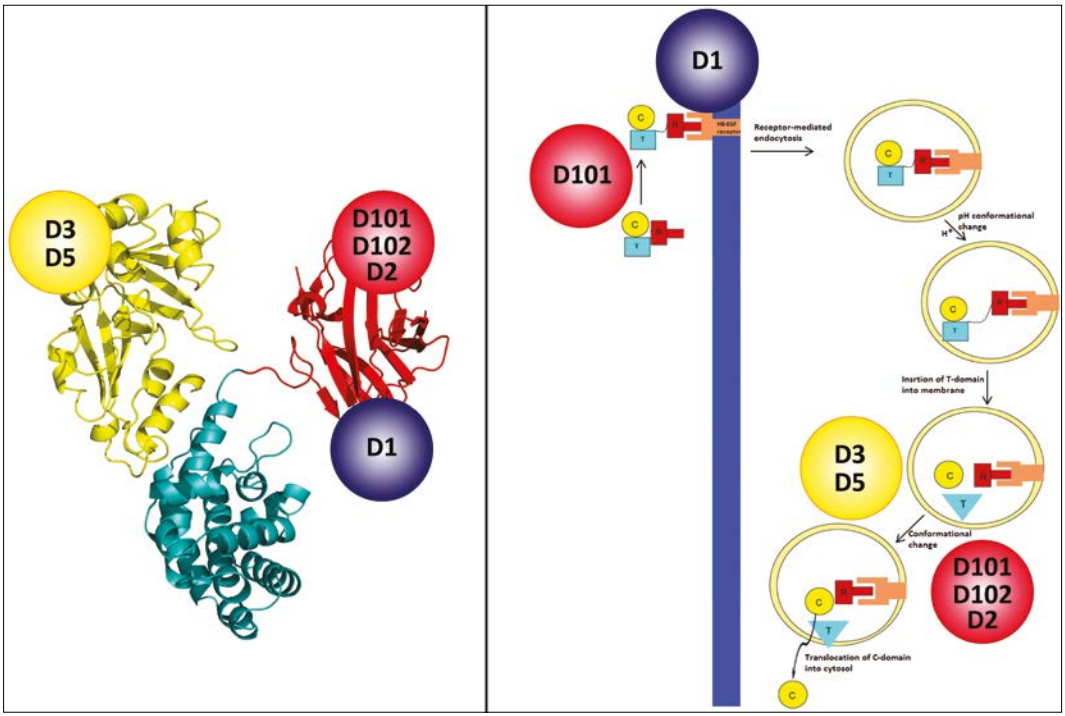


Figure 4

Left: cartoon representation of Diphtheria Toxin. The catalytic domain is in yellow, the translocation domain in cyan and the receptor binding domain in red. The binding sites of 6 antibodies are indicated by colored circles. Right: schematic representation of the cellular process leading to DT infection and cellular deaths. Colored circles with antibody names indicate the steps inhibited by antibody binding.

Investigating the role of Interleukin 3 and TIM3 for the detection and elimination of Acute Myeloid 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 (antibody) capable of discriminating them from normal, healthy cells. Such antibody 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)).

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 normal healthy cells with deleterious effects. We are attempting to overcome the problem with two complementary strategies. 1) We generated 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 singularly 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 might, as a consequence, discriminate leukemic from healthy cells.
2) As a proof of concept, we are investigating the correlation between the binding affinity of the antibody used as recognition element and its ability to discriminate healthy from cancer cells. Preliminary data suggests that appropriate tuning of the binding affinity increases the safety of our CAR system (less healthy cells killed) while not significantly compromising its efficacy (killing of leukemic cells).
In collaboration with Vadim Sumbayev (Uni. Kent) we have also been able to show that our anti-TIM3 antibody can effectively deliver a nanoparticle-drug conjugate to model cells overexpressing its target (TIM3 protein). Delivery to other cells is significantly less. We are repeating the experiment on ex-vivo AML cells obtained from human donors.
The drug used in our preliminary experiment is FDA approved for leukemic treatment but has limited use due to the high associated toxicity. We hope that our antibody-nanoparticle-drug conjugate might selectively deliver it to AML cells while sparing healthy ones. Even partial decrease of the interaction of the drug with healthy cells might have a significant effect on anti-AML treatment.
This work was done in collaboration with Ettore Biagi, Tettamanti Research Centre, Monza Hospital (IT) and Vadim Sumbayev, University of Kent (UK).

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.

Structural analysis and computational docking allowed us to design a mutated ER capable of binding EDCs with increased activity in comparison to the wild type protein. This mutated ER has been successfully produced and stabilized for a period of several months. We have shown that it can be used in EDC detection assays (SPR, fluorescence or mass spectroscopy based) with increased sensitivity in comparison to commonly used detection methods. Amplification of the SPR signal through the use of peptides that can recognize the ER conformational changes caused by EDC binding allowed the design of an SPR assays that can discriminate the presence of ER agonist or antagonist EDCs in a test sample.

This work was done in collaboration with Luigi Calzolai, European Union Joint Research Center, Ispra (IT) and Stephane Follonier, CSEM Landquart (CH).

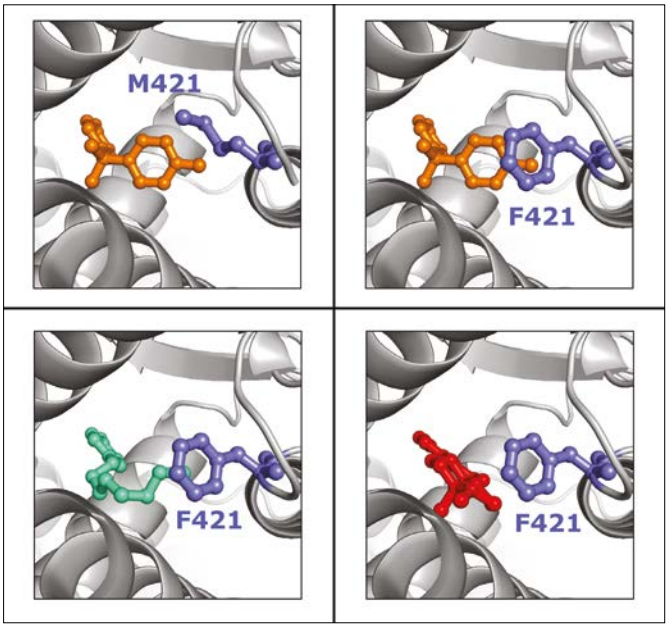


Figure 5
Details of the binding site of ER protein (grey cartoon) for EDC compounds. Selected EDC molecules (orange, green and red sticks) and their interaction with ER wild type (M421) or the rationally designed F421 ER mutant are shown in color.

Funding

European Union
RADAR: Rationally Designed Aquatic Receptors for the detection of organic pollutants
FP7- KBBE-2010-4-265721/2011-2014

Swiss National Science Foundation
Antibody-antigen interactions in Dengue virus
310030-138518-1 /2012-2015

Swiss National Science Foundation
Acquisition of a 600MHz solution NMR spectrometer
316030-157699
2014

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

Brazilian Swiss Joint Research programme
Antibodies against Dengue Virus: which ones are effective against this neglected disease and why? Searching for correlations between structural, dynamics and immunological properties of antibody/antigen complexes.
2012

Brazilian Swiss Joint Research programme – Seed Money
Antibodies against Dengue Virus: which ones are effective against this neglected disease and why? Searching for correlations between structural, dynamics and immunological properties of antibody/antigen complexes.
2014

Collaborations

CSCS, Swiss Supercomputer Center
Manno (CH)

BMRZ NMR Centre University of Frankfurt
Frankfurt (DE)

Adriano Aguzzi,
University of Zurich (CH)

Luigi Calzolai
European Union Joint Research Center, Ispra (IT)

Stephane Follonier
CSEM Landquart (CH)

Ana Paula Valente
University of Rio de Janeiro (BR)

Ettore Biagi
Tettamanti Research Centre, Monza Hospital (IT)

Vadim Sumbayev
University of Kent (UK)

Publications

Rationally Modified Estrogen Receptor Protein as a Bio-Recognition Element for the Detection of EDC Pollutants: Strategies and Opportunities.
Pedotti, M., V. E. Ferrero, T. Lettieri, P. Colpo, S. Follonier, L. Calzolai and L. Varani
Int J Environ Res Public Health. 2015; 12:2612-2621.

Epitope mapping by solution NMR spectroscopy.
Bardelli, M., E. Livoti, L. Simonelli, M. Pedotti, A. Moraes, A. P. Valente and L. Varani
J Mol Recognit. 2015;

The immune receptor Tim-3 mediates activation of PI3 kinase/mTOR and HIF-1 pathways in human myeloid leukaemia cells.
Prokhorov, A., B. F. Gibbs, M. Bardelli, L. Ruegg,

E. Fasler-Kan, L. Varani and V. V. Sumbayev
Int J Biochem Cell Biol. 2014; 59C:11-20.

Interleukin-1 beta induces the expression and production of stem cell factor by epithelial cells: crucial involvement of the PI-3K/mTOR pathway and HIF-1 transcription complex.
Wyszynski, R. W., B. F. Gibbs, L. Varani, D. Iannotta and V. V. Sumbayev
Cell Mol Immunol. 2014;

Rational modification of estrogen receptor by combination of computational and experimental analysis.
Ferrero, V. E., M. Pedotti, A. Chiado, L. Simonelli, L. Calzolai, T. Lettieri and L. Varani
PLoS One. 2014; 9:e102658.

Opening Study on the Development of a New Biosensor for Metal Toxicity Based on Pseudomonas fluorescens Pyoverdine.
Chiadò, A., L. Varani, F. Bosco and L. Marmo
Biosensors in Environmental Studies. 2013; 4:385-399.

Receptor-based high-throughput screening and identification of estrogens in dietary supplements using bioaffinity liquid-chromatography ion mobility mass spectrometry.
Aqai, P., N. G. Blesa, H. Major, M. Pedotti, L. Varani, V. E. Ferrero, W. Haasnoot and M. W. Nielen
Anal Bioanal Chem. 2013; 405:9427-9436.

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.

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.

Lectures and Seminars

Small Solutions for Big Water-Related Problems
Rome (IT) / 26-28.10.2014

Federal University of Rio de Janeiro (BR) / April 2014

Proteon User Meeting, Vienna (AT) / 10.2013

Bio-Rad Webinar / 09.2013

Politecnico Torino (IT) / 04.2013

University of Zurich / 02.2013

Scientific presentations to the general public

Lecture to elementary school students.
Monza (IT) / 02.2014

Lecture to Ticino elementary school students
Bellinzona (CH) / 07.2013

ASSOCIATE MEMBERS

SECTION 2

INDEX SECTION 2 – ASSOCIATE MEMBERS

Andrea Cavalli

Computational Structural Biology

Andrea Cavalli

Andrea Cavalli earned his degree in theoretical physics at the ETH in Zurich in 1995 and a Ph.D. 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., PNAS, 2007)) and the structural characterization of the intermediate state of a protein (Neudecker et al., Science, 2012). 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 overall objective of our research is to understand the role that structure and dynamics play in the definition of the function of biomolecules. In order to perform their function proteins, RNA and other biological molecules undergo a series of conformational changes that requires a precise balance between flexibility and stability. Changes in this equilibrium, induced by modifications such as genetic mutations, are often at the origin of diseases.

Novel and improved experimental techniques are starting to provide us with an increasing amount of data about structure and dynamics of biomolecules. Our aim is to develop accurate and mathematically sound methods to incorporate this data in computer simulations. We are particularly interested in the use of experimental data to extend the scope and accuracy of molecular dynamics simulations. This will enable us to study, at an atomistic level of details, complex processes such as molecular recognition, protein misfolding and aggregation.

Team

Associate Member: Andrea Cavalli, PhD > andrea.cavalli@irb.usi.ch
Members: Dariusz Ekonomiuk, PhD - Simon Olsson, PhD - Jacopo Sgrignani, PhD

Molecular characterization of a novel class of STAT3 inhibitors

Jacopo Sgrignani and Andrea Cavalli

Transcription factors (TFs) are central nodes in multiple oncogenic signaling pathways and represent attractive targets for development of novel cancer treatment strategies. However, very few direct pharmacological inhibitors of transcription factors are currently in the clinical trials. Signal Transducer and Activator of Transcription 3 (STAT3) belong to the STAT family of transcription factors. As other STAT members, STAT3 is a cytoplasmic protein and is regulated by multiple post-transcriptional modifications (PTM), like phosphorylation, methylation and acetylation.

Increased expression and activity of STAT3 is very common in human cancers. STAT3 has a central role in critical signaling pathways for tumor initiation and progression. STAT3 drives tumor progression by promoting proliferation, survival, metabolic adaptation, tumor angiogenesis and immune tolerance and its down-regulation by genetic or pharmacological means prevents or reverts tumorgenesis.

Many anticancer drugs inhibit upstream signaling pathways (e.g., JAK, EGFR) and affect STAT3 activation. In addition to these "indirect" inhibitors of the STAT3 pathway (e.g., JAK inhibitors), there is increasing interest in developing "direct" inhibitors of STAT3 that might interfere with the multiple diverse functions of this TF.

A number of small molecule compounds as well as natural products have been identified as direct STAT3 inhibitors (STAT3i). The aim of this study is to investigate the mechanism of action of a novel class of compounds with STAT3 inhibitory activity. In particular, we will study two compounds that interfere effectively with STAT3 and have potent anticancer activity in various tumor models. Experimental results suggest, that this new class of compounds acts by promoting the formation of large aggregates of STAT3 and that the formation of this aggregates is a direct consequence of conformational changes, disruption of specific inter-domain interactions and partial unfolding of STAT3 induced by STAT3i.

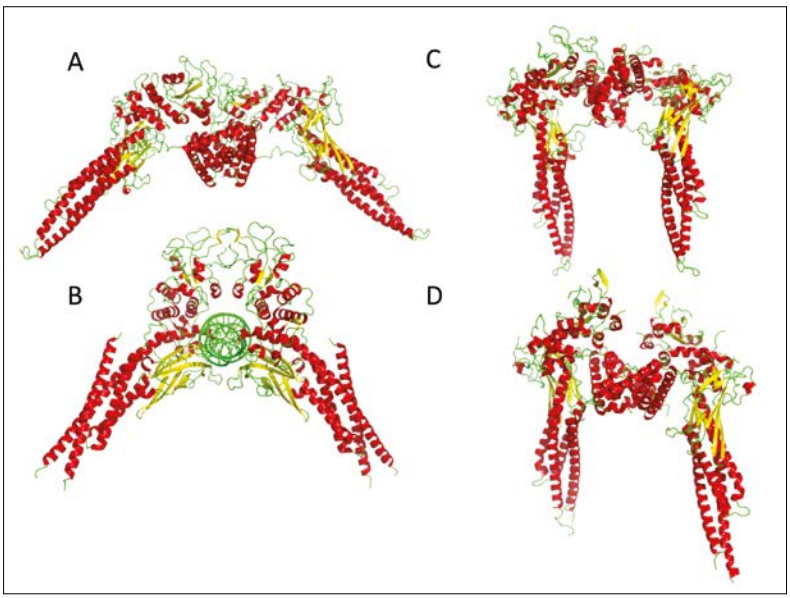


Figure 1
Computational models of the STAT3 homodimer.

Study Objective

The objective of this study is the characterization of the mechanism of action of STAT3i at a molecular level. In particular we aim to:

– Investigate the effect of small molecule drug binding on the stability of inter-domain interactions and the mobility of STAT3 domains.
– Investigate the effect of changes in stability and mobility of STAT3 domains on the formation of aggregates and its role in STAT3 inactivation.

Identification of potential determinants of immunoglobulin light chain amyloidosis

Dariusz Ekonomiuk and Andrea Cavalli

Light chain amyloidosis is a disorder associated with aggregation of immunoglobulin (Ig) light chains. Ig light chains with different sequences reveal varied amyloidogenic propensities and it is currently not clear which factors drive fibrillation process and, thus, cause pathological conditions.

In this project, we investigate a repertoire of toxic and non-toxic sequences and perform molecular dynamics simulation for selected light chain models with different amyloidogenic propensities in order to identify the molecular determinants of light chain amyloidosis.

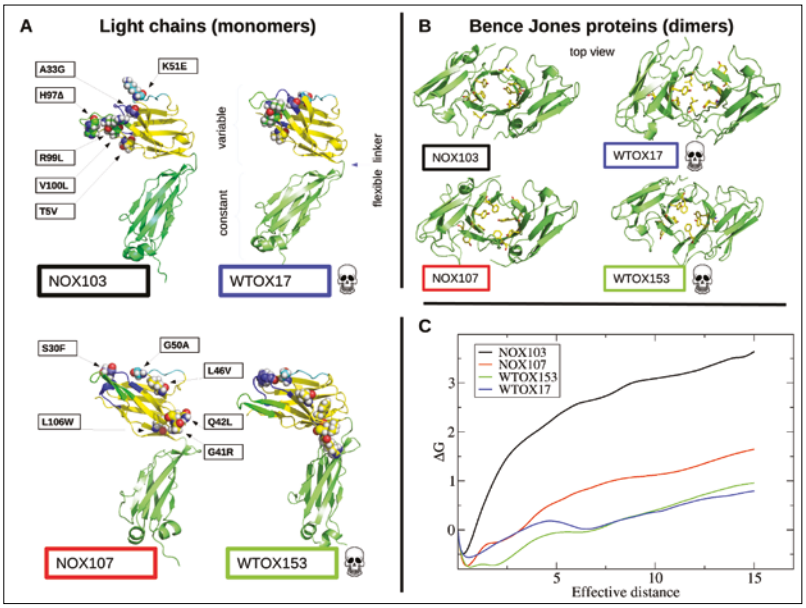


Figure 2
A) Mapping differences between toxic (WTOX17 and WTOX153) and nontoxic (NOX03 and NOX107) LC models: Constant domain is depicted in lime green; variable domain framework regions are in yellow; complementarity determining regions (CDRs): CDR1, CDR2 and CDR3 are represented in blue, cyan, and green, respectively. B) Bence Jones protein models: Top view of the variable domain of two toxic and two nontoxic LCc. C) Differentiation of BJ protein interface stability by energetic evaluation: The toxic light chains (green and blue plots) have lower inter-chain interface stability than nontoxic LCs (black and red plots).

Characterization of toxic oligomer present in Alzheimer's disease-associated amyloid fibrillation process

Dariusz Ekonomiuk and Andrea Cavalli

Alzheimer's disease (AD) is recognized as the most spread neurodegenerative disease affecting over 30 million people worldwide. The development of the disorder has been linked to the presence of extracellular beta-amyloid (Ab) peptide aggregates of different sizes. Ab oligomeric species formed at early stages of the aggregation process are leading candidates for causing AD. Thus, targeting oligomers can be a very valuable strategy to combat Alzheimer's disease. However, the molecular mechanism underlying the self-assembly of the different Ab species is not fully understood and it is not clear how the early soluble oligomeric species associate to form protofibrils and, subsequently, mature fibrils.
The main objective of this project is to apply computational techniques to elucidate small angle X-ray scattering (SAXS) data collected by our collaborators at University of Cambridge (Prof. M. Vendruscolo group) and resolve major coexisting components in Ab fibrillation process.

Understanding the molecular details of catalysis in a proline isomerase

Simon Olsson and Andrea Cavalli

Cyclophilins are a part of the ubiquitous family of enzymes which catalyses the isomerisation transition between peptidyl and prolyl conformations, which plays a crucial role in the folding of many proteins. However, these enzymes have also been identified as a putative drug-target to treat a number of diseases, including viral infections such as Hepatitis C. In this project we wish to understand the molecular details of the catalysis of Cyclophilin A, and in particular also the inhibition of this function. To this end, we are collaborating with the group Prof. Riek at the ETH in Zurich to analyze high-resolution exact nuclear Overhauser enhancement data and residual dipolar coupling data on Cyclophilin A in complex with cyclosporin, which is known inhibitor of its function, and in absence of this inhibitor.

In silico equilibrium protein folding experiments

Simon Olsson and Andrea Cavalli

The process of how proteins reach their native basin of structures is poorly understood and constitutes an important problem in molecular biology. This process is called the protein folding problem, and is generally thought to proceed through large, concerted changes in structure. In this project, we are studying the folding of two small proteins: the WW domain of Pin1 and Porcine peptide YY. These studies are carried out using molecular simulation combined with exact nuclear Overhauser enhancement data and/or chemical shift data obtained at multiple temperatures measured in the groups of collaborators Prof. Riek at the ETH in Zürich or Prof. Zerbe University of Zürich. Specifically, we are integrating all the experimental data with one simulation to obtain a full, thermodynamic and structural description of the protein folding process.

Funding

Tropos Stiftung für humane Verhaltensforschung
Determination of the free energy landscapes of proteins: application to Alzheimer's disease
2013-2014

Collaborations

Michele Vendruscolo
University of Cambridge (UK)

Sonia Longhi
University of Aix-Marseille I (FR)

Jose Rizo-Rey
University of Texas Southwestern Medical Center, Dallas (US)

Publications

Characterization of the Conformational Fluctuations in the Josephin Domain of Ataxin-3.
Sanfelice, D., A. De Simone, A. Cavalli, S. Faggiano, M. Vendruscolo and A. Pastore
Biophys J. 2014; 107:2923-2931.

A probabilistic approach to the determination of native state ensembles of proteins.
Olsson, S., B. R. Vögeli, A. Cavalli, W. Boomsma, J. Ferkinghoff-Borg, K. Lindorff-Larsen and T. Hamelryck
J. Chem. Theory Comput. 2014; 10:3484-3491.

Archaeal MBF1 binds to 30S and 70S ribosomes via its helix-turn-helix domain
F. Blombach, H. Launay, A. P. Snijders, V. Zorraquino, H. Wu, B. de Koning, S. J. Brouns, T. Ettema, C. Camilloni, A. Cavalli, M. Vendruscolo, M. J. Dickman, L. D. Cabrita, A. La Teana, D. Benelli, P. Londei, J. Christodoulou, J. van der Oost in Biochem J (2014)

ALMOST: An all atom molecular simulation toolkit for protein structure determination.
Fu, B., A. B. Sahakyan, C. Camilloni, G. G. Tartaglia, E. Paci, A. Caflisch, M. Vendruscolo and A. Cavalli
J Comput Chem. 2014; 35:1101-1105.

Determination of the individual roles of the linker residues in the interdomain motions of calmodulin using NMR chemical shifts.
Kukic, P., C. Camilloni, A. Cavalli and M. Vendruscolo
J Mol Biol. 2014; 426:1826-1838.

A conformational ensemble derived using NMR methyl chemical shifts reveals a mechanical clamping transition that gates the binding of the HU protein to DNA.
Kannan, A., C. Camilloni, A. B. Sahakyan, A. Cavalli and M. Vendruscolo
J Am Chem Soc. 2014; 136:2204-2207.

Replica-Averaged Metadynamics.
Camilloni, C., A. Cavalli and M. Vendruscolo
J. Chem. Theory Comput. . 2013; 12:5610-5617.

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

The imaging facility is central to most of the research projects. It includes the flow cytometry and microscopy services. The state-of-the-art *flow cytometry* facility 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. Rocco D'Antuono joined the IRB in 2014 and is responsible for the microscopy facility and the image analysis. He has experience in confocal microscopy, high-content cellular analysis and multiphoton microscopy.

Flow cytometry

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-art multicolor flow cytometry instruments. Cell sorting is performed on a FACSAria III 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 25,000 events/sec) with high-purity (up to 99%).

Benchtop analysers: The Flow cytometry lab is equipped with an advanced benchtop analyzer BD Fortessa equipped with four lasers (488, 561, 640 and 405 nm excitation wavelengths) and 16 fluorescence channels, one FACSCantoII (488, 640 and 405 nm lasers-eight colors) and FACSCanto I (488 and 640 nm lasers-six colors). The lab also acquired a SP6800, a Spectral Cell Analyzer from Sony (405, 488 and 640 nm lasers excitation wavelengths) equipped with 34 channel PMT collecting spectra of all emitted lights with no need for conventional bandpass filters.

High throughput screening: Beside both FACSCanto equipped with HTS for acquisition of 96 and 384 wells plates, the lab has also been equipped with an Intellicyt (488 and 633 nm excitation-four colors) with automated platform (up to 45 plates per run) allowing high throughput screening for 96 or 384 well plates format. The flow laboratory offers efficient support and high quality instruments.
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

The main Microscopy Facility is equipped to perform most of the procedures for cell and tissue imaging (including FRET, FRAP, live cell imaging and intra-vital microscopy); it also offers support for sample preparation, image analysis, deconvolution and 3D reconstruction; thanks to a wide range of software such as MetaMorph (Molecular Devices), Matlab (MathWorks), Imaris (Bitplane), ImageJ and CellProfiler.
The instrumentation includes wide-field fluorescence microscopes, high-content imaging system for image acquisition and analysis, confocal and multiphoton microscopes, comprising a surgical area for intra-vital microscopy:

- Confocal microscope. *Leica TCS SP5*, equipped with 4 new-generation hybrid detectors (*HyD* Leica technology) and aberration-corrected objectives for high-resolution imaging (e.g. 100X 1.4 Oil) (Figure 1).
- High-content screening system. BD *Pathway 855*, wide-field or confocal automated microscope, equipped with *Twister II Plate Handler (Caliper)*, allowing acquisition and analysis of up to 35 plates.
- Wide-field microscopes.
  - *Nikon Eclipse E800* upright microscope;
  - *Nikon Eclipse TE300* inverted microscope, with incubator for live-cell experiments and *Eppendorf FemtoJet* microinjector;
  - *Zeiss Axiovert 200* inverted microscope, equipped with UV-corrected optics and *TILLvisION* software for calcium measurement experiments.
- Surgical microscopes.
  - *Olympus SZX10* equipped with a *DP80* dual CCD color and monochrome camera for the imaging of tissues and fluorescence during the surgery.
  - *Leica M651* equipped with a *MC170 HD* color camera.
- Multi-Photon Excitation microscopy system. *LaVision BioTech TriM Scope*, assembled system with upright and inverted microscopes, equipped with two tunable pulsed NIR lasers and OPO for multi-colour simultaneous imaging. Fluorescence is detected using new-generation hybrid detectors and infrared-corrected objectives, including a microendoscope (Figure 2).

Figure 1 *Left: Confocal microscope Leica TCS SP5. Right: High-content screening system BD Pathway 855.*

Figure 2 *Two-photon excitation microscopy system: view of inverted and upright microscopes.*

Gene Expression and Protein Production Facility

The IRB has developed relevant competences in the field of protein-protein and antigen-antibody interaction applied to the study of infectious and inflammatory diseases. The Gene Expression and Protein Production Facility (GEPP) focuses primarily on biochemical and biophysical methods to support research projects in the field of infectious diseases and immunology. Our principal interest focuses on vaccine discovery and host pathogen interaction. We have developed a strong platform to produce, purify and analyze complex viral antigens from mammalian and insect cells. We provide assistance and information to all IRB researchers on chemical and physical methods applied to biological samples. We have collaborations in place for routine sample analysis by mass spectrometry and electron microscopy.

We can provide training and help in biophysical analysis of macromolecules by Circular Dichroism (CD), Dynamic Light Scattering (DLS) and High-Performance Liquid Chromatography (HPLC). We are also equipped to investigate protein/protein and protein/molecule interactions by Isothermal Titration Calorimetry (ITC) and/or Surface Plasmon Resonance (SPR). We also have experience in mice monoclonal antibodies generation and can help with the design of experiment and hybridoma generation. Our current focuses are on human cytomegalovirus (HCMV) vaccine development and human respiratory syncytial virus (RSV) vaccine development.

The team currently collaborates with academic centers and industry and will consider all collaborations based on their rational, potential and biomedical interest.

Kabanova, A., et al. *
Proc Natl Acad Sci U S A. 2014, 111(50): 17965-17970.

Corti, D., et al. *
Nature. 2013, 501(7467):439-443.

PhD PROGRAMME
SECTION 4

INDEX SECTION 4 – PhD PROGRAMME

Camilla Basso

The role of IL-1β and myeloid cells in the generation of multifunctional pathogenic T helper cells in experimental autoimmune encephalomyelitis

Supervisor: Antonio Lanzavecchia, Federica Sallusto // Co-referees: Carole Bourquin, Annette Oxenius
Swiss Federal Institute of Technology, Zurich, Switzerland

IL-1β is a pleiotropic cytokine that plays a role in several inflammatory disorders in humans and in experimental animal models, including mouse experimental autoimmune encephalomyelitis (EAE). IL-1β is produced after pro-IL-1β cleavage by IL-1 converting enzyme (caspase-1), which in turn is activated by a complex of proteins called inflammasome. IL-1β drives human Th17 cells polarization and triggers the differentiation of mouse inflammatory Th17 cells characterized by the co-expression of IL-17 and IFN-γ. In the study presented in this thesis, it is reported that mice deficient for IL-1β production or for a component of the inflammasome (the apoptosis-associated speck-like protein containing a caspase recruitment domain, also known as ASC) do not develop EAE following immunization with myelin oligodendrocyte glycoprotein (MOG), emulsified in complete Freund's adjuvant (CFA), and pertussis toxin (PT) administration. Autoreactive T cells were primed in both 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, the T cells expanded poorly and showed reduced capacity to produce simultaneously several inflammatory cytokines, in particular GM-CSF. Interestingly, generation of multifunctional (IL-17+, IL-22+, IFN-γ+, GM-CSF+) T cells in wt mice required the presence of PT at the time of immunization. PT was found to rapidly induce IL-1β secretion by CD11c+ and Gr1+ myeloid cells in secondary lymphoid organs in vivo, and by bone-marrow-derived dendritic cells (BM-DCs) in vitro. Moreover, in mice where Gr1+ myeloid cells were depleted or in mice with impaired migration of monocytes due to the deficiency in the chemokine receptor CCR2 (binding the monocyte chemoattractant protein-1, MCP-1), IL-1β production was not induced by PT and multifunctional T cells priming was impaired. When T cells were primed in mice deficient for myeloid differentiation primary response protein 88 (MyD88) encoding gene, the PT-driven recruitment of inflammatory myeloid cells in the lymph node and the generation of multifunctional T cells were also impaired. Moreover, selective deletion of MyD88 in T cells was sufficient to block the PT-induced differentiation of multifunctional Th17 cells. Taking together, these data support the notion that the disease-inducing effect of PT is due to its ability to determine recruitment of myeloid cells, production of IL-1β and differentiation of highly pathogenic multifunctional T cells.

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

***Mele F., et al.** **
Nat. Commun. 2015, 6:6431.

Simone Becattini

Diversity of the human memory T cell repertoire to pathogens and vaccines

Supervisor: Antonio Lanzavecchia // Co-referees: Salomé Leibundgut-Landmann, Federica Sallusto
PhD Program in Science de la vie, University of Lausanne, Switzerland

Human CD4+ T cells are key players in orchestrating the response to pathogens and vaccines. Upon first antigen encounter, naïve T cells get activated and clonally expand, meanwhile acquiring effector functions to control the threat and later becoming memory T cells to assure appropriate response in case of re-challenge. Different classes of pathogens are known to induce distinct polarized T cell subsets, each characterized by specific homing and functional properties, that can mount the most efficient response tailored to the challenging pathogen. Thus, viruses and intracellular bacteria induce IFN-γ-producing Th1 cells while helmints induce IL-4-producing Th2 and fungi and extracellular bacteria induce IL-17-producing Th17 cells. Once considered terminally differentiated stages, polarized T cells can show remarkable flexibility and have been proven to undergo phenotypic switch or divergent differentiation in several experimental systems. Understanding the generation of diversity in the human T cell memory compartment would have enormous implications both on a biological and on a clinical level. Using cell sorting of human memory Th1, Th2, and Th17 cells followed by CFSE labeling, antigenic stimulation, and next generation TCR Vβ sequencing, we were able to demonstrate that memory T cells specific for pathogens such as Candida albicans and Mycobacterium tuberculosis or tetanus toxoid vaccine could be present in all subsets, albeit at different frequencies. Interestingly, several clonotypes were present in more than one subset and, in some cases, even in all subsets, while other clonotypes were restricted to one particular subset. By cloning antigen-specific T cells from memory subsets we were able to isolate several T cell clones from Th1, Th2, and Th17 subsets and show that they share the same TCR but display different transcription factors, cytokine production and chemokine receptor expression, characteristic of the subset from which they were isolated. Collectively, these results indicate that the T cell response to pathogens and vaccines can comprise T cell clones that in spite of identical TCR, display different, sometimes divergent types of effector functions and provide for the first time demonstration of intraclonal diversification in the human T cell response.

* ***Engen S.A., et al.*** *PLoS One 2014, 9:e104306.*

* ***Kabanova A., et al.*** *Proc. Natl. Acad. Sci. USA 2014, 111:17965-17970.*

* ***Becattini S., et al.*** *Science 2015 347:400-406.*

* ***Mele F., et al.*** *Nat. Commun. 2015, 6:6431.*

Tess Melinda Brodie

CD4 T cell subsets: phenotypic, functional and repertoire analysis at steady state and allergic inflammation

Supervisor: Federica Sallusto // Co-referees: Burkhard Becher, Christian Muenz, Mario Roederer
University of Zurich, Switzerland

In my thesis I performed two studies. In the first study, I developed a protocol for in-depth phenotypic analysis of T cell subsets with flow cytometry. Despite the long held importance of chemokine receptor (CKR) expression in identifying subsets, nothing had been published as a method to discriminate many diverse populations based on CKR expression with flow cytometry. To perform a multiparametric analysis on fresh PBMC, we developed a flow cytometry monoclonal antibody panel, did many quality control experiments to optimize it, and published it for other labs with similar interests. This panel allows for the identification of 9 different CKRs at the same time within CD4 or CD8 T cells. With analysis programs like Pestle and Spice, one can study all possible combinations of these receptors within T cell subsets and check for differences between healthy and pathological states. We used this tool in a second study to compare differences in CKR expression in CRTh2 cells in allergic and non-allergic donors. In this study I had two main purposes: identification of the CD4 Th2 subset most enriched in allergen specific cells in allergic individuals and determination of the phenotype of IL-9 producing cells in allergy. T cell repertoire analysis (using the T cell library method discussed in methods) revealed that CRTh2 expression identifies T cells that not only have a higher frequency of allergen specific cells in allergic donors, but these cells have higher functional avidity for allergen, respond more to immunodominant epitopes in allergens and correlate best with IgE to allergen. In response to allergens, CRTh2 cells made Th2 cytokines, IL-4, IL-5, IL-13, as well as IL-9. Recent publications show that IL-9 is not secreted by IL-4 producing cells, but by Th9 cells that make no IL-4 (49, 128). Our analysis demonstrated that a single CRTh2 cell is capable of making all 4 cytokines in response to allergen and that these cells are phenotypically Th2 cells.

***Oseroff C., et al.** **
J. Immunol. 2012, 189:1800-1811.

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

***Mahnke Y.D., et al.** **
Eur. J. Immunol. 2013, 43:2797-2809.

***Campion S.L., et al.** **
J. Exp. Med. 2014, 211:1273-1280.

Marie-Luise Humpert

Functional role of CXCR7 on late germinal center B cells and molecular mechanisms of CXCR7 sorting

Supervisor: Marcus Thelen // Co-referee: Marlene Wolf
Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland

Chemokines and chemokine receptors promote migration of leukocytes, endothelial and epithelial cells and function in homeostasis and inflammation. Chemokine receptors are seven transmembrane domain G-protein coupled receptors that bind chemokines with high affinity and thereby activate different signaling pathways leading to chemotaxis, secretion and transcriptional activation. Atypical chemokine receptors differ from these receptors by their inability to trigger G-protein mediated signal transduction, but instead scavenge their chemokine ligands by rapid cycling between plasma membrane and endosomes. The scavenger receptor CXCR7 was reported to mediate signals through binding to β-arrestin2, which leads to internalization of the receptor. But most likely the main function of CXCR7 is sequestration of CXCL12 and CXCL11. The expression of CXCR7 on non-hematopoietic cells and neoplasms is widely accepted, however, its expression on leukocytes has been challenged.

This thesis unveils a functional expression of the receptor in mouse and human B cells by using combined molecular and proteomic approaches. Determination of receptor surface expression on tonsillar mononuclear cells (TMCs) revealed that CXCR7 is expressed on CD19+CD27+ memory B cells, on CD19+CD38+CD138- and intracellular immunoglobulin (icIg) containing plasmablasts, but not on CD19+CD138+ plasma cells. By using an in vitro differentiation system, which mimics B cell maturation from the memory B to the plasma cell stage, a marked inverse correlation of CXCR7 and CXCR5 cell surface levels could be shown. Moreover transwell migration assays performed with TMCs or in vitro differentiated cells pointed out that inhibition of CXCR7 markedly increases chemotaxis towards CXCL12, especially at late stages of B cell maturation. Taken together the findings unequivocally demonstrate an important role of CXCR7 in regulating the migration of plasmablasts and plasma cells.

In a second project ligand-independent and -dependent receptor trafficking was studied to elucidate the mechanism of chemokine scavenging. With the aid of receptors tagged fluorescently at their N-terminus and fluorescent ligands the initial steps of cargo sorting were investigated.

* ***Humpert, M.L. et al.***
Proteomics 2012, 12:1938-1948.

* ***Humpert, M.L., et al.***
Eur. J. Immunol 2014, 44:694-705.

Jessica Merulla

Deciphering the complexity of mammalian ER quality control and ERAD pathways

Supervisor: Maurizio Molinari // Co-referees: Peter Bütikofer, Mario Tschan
University of Bern, Switzerland

Only native polypeptides are released from the endoplasmic reticulum (ER) to be transported at the site of activity. Persistently misfolded proteins are retained and eventually selected for ER-associated degradation (ERAD). The paradox of a structure-based protein quality control is that functional polypeptides may be destroyed if they are architecturally unfit. This has health threatening implications as shown by the numerous "loss-of-function" proteopathies, but also offers chances to intervene pharmacologically to promote bypassing of the quality control inspection and export of the mutant, yet functional protein. Here we challenged the ER of human cells with 4 modular glycopolypeptides designed to alert luminal and membrane protein quality checkpoints. Our analysis reveals the unexpected collaboration of the cytosolic AAA-ATPase p97 and the luminal quality control factor UDP-glucose:glycoprotein glucosyltransferase (UGGT1) in a novel, BiP- and CNX-independent checkpoint. This prevents Golgi transport of a chimera with a native ectodomain that passes the luminal quality control scrutiny, but displays an intramembrane defect. Since human proteopathies may result from impaired transport of functional polypeptides with minor structural defects, identification of quality checkpoints and of treatments to bypass them as shown here upon silencing or pharmacologic inhibition of UGGT1 or p97, may have important clinical implications.

***Merulla, J., et al.** **
Traffic 2013, 14:767-777.

***Merulla, J., et al.** **
Mol. Biol. Cell 2015, 26:1532-1542. (Recommended by the Faculty of 1000)

Julia Noack

Mechanisms regulating the recovery from transient endoplasmic reticulum stress in mammalian cells

Supervisor: Maurizio Molinari // Co-referees: Markus Aebi, Ari Helenius, Ulrike Kutay, Roberto Sitia
Swiss Federal Institute of Technology, Zurich, Switzerland

Eukaryotic cells respond to changes in ER homeostasis by inducing transcription/translation of endoplasmic reticulum (ER)-resident gene products in a series of tightly regulated events collectively named the unfolded protein response (UPR). The enhanced luminal content of molecular chaperones, folding and ERAD factors reduces the burden of unfolded and misfolded polypeptides in the ER lumen and re-establishes proteostasis (i.e., the organellar capacity to produce appropriate quality and quantity of the cellular proteome). In yeast, the expansion of the ER during ER stress is counterbalanced by ATG-independent, lysosomal degradation of the ER (ER-phagy) and this mechanism is crucial for the survival of the cell. It is, however poorly -if at all- described by which mechanisms the luminal content and the size of the ER return to the initial state in the recovery phase after a transient stress in mammalian cells. In most reports, special emphasis has been laid on transcriptional events and their regulation, but much less is known about the regulation of the levels of the actual mediators of these functions, namely the ER stress-induced proteins. Here we show that whereas stress induced transcripts decay at similar rates, kinetics regulating return of ER stress-induced chaperones at their pre-stress levels are highly divergent and require intervention of both the proteasome and of reticulophagy.

***Bernasconi, R., et al.** **
Autophagy 2012, 8:1534-1536.

***Noack, J., et al.** **
Autophagy 2014, Hayat, M.A. Ed. Elsevier, Oxford, Vol 2:129-136.

***Noack, J., et al.** **
J. Virology 2014, 88:10272-10275.

***Noack, J., et al.** **
Swiss Medical Weekly 2014, 144:w14001, 1-13.

***Noack, J. and Molinari, M.** **
Nature Chem. Biol. 2014, 10:881-882.

Leontios Pappas

The role of germline residues and redundant somatic mutations in the development of broadly influenza neutralizing antibodies

Supervisor: Antonio Lanzavecchia // Co-referees: Annette Oxenius, Antonius Rolink, Federica Sallusto
PhD Program in Science de la vie, University of Lausanne, Switzerland

Influenza virus is responsible for 3.5-5 million cases of severe disease and 300,000-500,000 deaths annually. The neutralizing antibody response to influenza is dominated by antibodies that bind to the globular head of the hemagglutinin (HA), which undergoes a continuous antigenic drift, necessitating the re-formulation of influenza vaccines on a yearly basis. Recently, several laboratories have described a new class of rare influenza-neutralizing antibodies that target a conserved site in the HA stem. Although such broadly neutralizing antibodies have the capacity for effectively neutralizing multiple different strains of the virus, to date there is little knowledge on how these antibodies develop. This study has attempted to address this issue by dissecting the evolutionary pathways broadly influenza neutralizing antibodies follow as a result of affinity maturation.

Most anti-HA stem antibodies use the heavy chain variable region VH1-69 gene, and structural data demonstrate that they bind through conserved heavy chain complementarity determining region (HCDR) residues. However, the VH1-69 antibodies are highly mutated and are produced by several but not all individuals suggesting that several somatic mutations may be required for their development. By analyzing 197 anti-stem antibodies from a single donor, the developmental pathways of several VH1-69 clones were reconstructed and two key elements that are required for the initial development of most VH1-69 antibodies were identified: a polymorphic germline-encoded F at position 54 and a conserved Y at position 98 in the HCDR3. Strikingly, in most cases a single P52aA mutation in the HCDR2 was sufficient to confer high affinity binding to the selecting H1 antigen, consistent with rapid affinity maturation. Surprisingly, additional favorable mutations continued to accumulate, increasing the breadth of reactivity and making the initial mutations, and even F54, redundant. These results define VH allele polymorphism, the VDJ rearrangement and single somatic mutations as the three requirements for the generation of broadly neutralizing VH1-69 antibodies, and reveal an unexpected redundancy in the affinity maturation process.

By reconstructing the genealogy trees of several influenza-specific human B cell clones two distinct evolutionary pathways leading to broadly VH1-69 influenza neutralizing antibodies were identified. Those bearing Y98 in their HCDR3 preferentially accumulate a P52aA/G mutation in the HCDR2. In contrast, antibodies without Y98 do not mutate the germline P52a residue and follow maturation pathways via distinct substitutions in the HCDR1. Finally, this study elucidates the definitive role of the HCDR3 in determining whether different amino acid substitutions have a positive or neutral role in antigen binding on a particular antibody scaffold. This finding is especially relevant for identifying substitutions that may confer broad binding reactivity. The broadly neutralizing CR9114 antibody requires 8 amino acid substitutions in order to bind to Influenza A group 1 and 2 and Influenza B strains, which do not necessarily confer the same breadth of binding on different HCDR3 scaffolds. Taken together, these results demonstrate that the HCDR3 is important not only for defining the antigen binding properties of naïve B cells, but it also constrains the maturation pathways a clone may follow to achieve broad, high affinity binding.

This work has significantly advanced our understanding of how antibodies target the HA stem, by identifying the substitutions selected to confer binding as the result of antigen-driven affinity maturation in the human antibody response. Such knowledge is critical for informing the design of HA stem-based immunogens capable of eliciting antibodies of similar breadth and potency. Potent, broadly neutralizing antibodies play a key role for informing structurally-guided vaccine design, which has recently been successfully applied for determining a promising vaccine candidate for Respiratory Syncytial Virus. We hope that the novel findings on the maturation process of broadly neutralizing anti-stem antibodies presented in this study may eventually assist in the development of a universal influenza vaccine.

Pappas L. et al. **Nature 2014, 516:418-422*

Luca Piccoli

Origin and pathogenicity of GM-CSF autoantibodies in patients with pulmonary alveolar proteinosis

Supervisor: Antonio Lanzavecchia // Co-referees: Federica Sallusto, Manfred Kopf
Swiss Federal Institute of Technology, Zurich, Switzerland

A fundamental property of the adaptive immune system is the ability to generate an extraordinarily large number of different receptors. In particular, antibody diversity is generated at two distinct stages of B cell development: in bone marrow by rearrangement of the VDJ genes and in germinal centers by somatic mutations. Both processes have the potential to generate self-reactive B cells that, when activated, can produce autoantibodies and, depending on their specificity, cause an autoimmune disease.

Piccoli L. et al **Nat. Commun. 2015, 6:7375.*

The aim of my thesis is to understand the pathogenesis of autoimmune pulmonary alveolar proteinosis (PAP) and the origin of autoantibodies to granulocyte-macrophage colony stimulating factor (GM-CSF) that cause this severe autoimmune disease. By immortalizing memory B cells, I isolated a panel of anti-GM-CSF monoclonal autoantibodies from PAP patients and identified the epitopes recognized. By measuring GM-CSF neutralization, I found that three non-cross-competing autoantibodies sequester GM-CSF in stable immune complexes and completely neutralize the cytokine activity in vitro, while in vivo the same immune complexes are rapidly cleared in a Fc-dependent fashion. In contrast, single autoantibodies could only partially neutralize GM-CSF activity in vitro, depending on the stoichiometry of the assay, and enhanced the levels of bioavailable GM-CSF in vivo. These findings provide a plausible explanation for the severe phenotype of PAP patients that developed high levels of GM-CSF autoantibodies to multiple epitopes of the molecule forming immune complexes that mediate GM-CSF sequestration and degradation.

To investigate the origin of autoantibodies, I removed somatic mutations from seven anti-GM-CSF antibodies to produce the "unmutated common ancestor" (UCA), i.e. the antibody produced by the naïve B cell that gave origin to the autoantibody. In 5 out 7 cases, the UCA of anti-GM-CSF antibodies were not able to bind to GM-CSF, while in the remaining 2 cases there was a very low but still detectable binding. Similarly, the UCA of a high-affinity autoantibody isolated from a patient with rheumatoid arthritis and specific for citrullinated vimentin failed to bind to the self antigen. In contrast, the UCA of anti-influenza hemagglutinin (HA) antibodies bound HA with similar or only slightly reduced affinity. Taken together, these findings are consistent with a model where self-reactivity of B cells is acquired through somatic mutations during the response to a foreign antigen.

Silvia Preite

On the regulation of antibody responses by T follicular helper cells

Supervisors: Federica Sallusto, Facundo Batista // Co-referees: Francesca Granucci, David Gray
International PhD Course in Molecular Medicine, Università Vita-Salute San Raffaele, Milan, Italy

Cognate interaction between T helper cells and B cells is required for production of high-affinity antibodies and the generation of memory B cells and long-lived plasma cells. In this PhD work, I studied the impact of T cell help on humoral immune responses. I used an adoptive T cell transfer system, whereby low numbers of antigen-specific T cells were transferred into T cell-deficient hosts and serum antibody levels, germinal center reaction, as well as generation of antibody-secreting cells and long-lived plasma cells were tracked at different time points after immunization. I found that, upon immunization, transferred CD4+ T cells proliferated extensively in lymphopenic host, differentiated preferentially into T follicular helper (Tfh) cells that provided help for induction of germinal center (GC) B cells and a strong primary antibody response. Maintenance of Tfh cells required sustained antigenic stimulation through cognate interactions with GC B cells. In spite of the exuberant Tfh and GC B cell response, serum antibody levels were not sustained and booster immunization failed to induce secondary antibody response. Although immunoglobulin class-switch recombination, initiation of GC reaction, and differentiation of antibody-secreting short-lived plasma blasts were normal, affinity maturation and somatic hypermutation were impaired and high-affinity long-lived plasma cells were not detectable in the bone marrow. Moreover, in lymphopenic conditions, a strong and prolonged Tfh cell response led to bystander B cell activation, hyper-gammaglobulinemia, and production of poly- and self-reactive antibodies. Importantly, transfer of T regulatory (Treg) cells in lymphopenic mice before immunization restricted Tfh cell proliferation and restored normal B cell responses, including affinity maturation. Taken together, this work identifies critical steps in the regulation of Tfh-induced antibody responses and provides support to the notion that dysregulated Tfh cell responses can lead to development of autoantibodies.

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

* ***Kabanova A., et al.*** *Proc. Natl. Acad. Sci. USA 2014, 111:17965-17970.*

PhD LECTURE COURSE & SEMINARS

SECTION 5

INDEX SECTION 5 – PhD LECTURE COURSE & SEMINARS

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

PhD Lecture Course 2012 – 2013

Robert Tampé
"Mechanism of the MHC I Peptide Loading Complex in Adaptive Immunity"
Goethe University Frankfurt, Frankfurt (DE) / 19.10.2012

Andrew McMichael
"T cell control of HIV-1 in acute infection"
Weatherall Institute of Molecular Medicine, University of Oxford, Oxford (UK) / 19.11.2012

Dan Littman
"Th17 cell differentiation and role in autoimmunity: role of transcription factors and commensal bacteria"
New York University School of Medicine, New York (US) / 18.12.2012

Ritta Lahesmaa
"Molecular mechanisms of human T cell differentiation to functionally distinct subsets"
University of Turku and Åbo Akademi University, Turku (FN) / 21.01.2013

Maria Rescigno
"Players of intestinal immune homeostasis"
European Institute of Oncology, Milan (IT) / 26.02.2013

Ronald N. Germain
"How Imaging Has Revealed the Central Role of Cell Dynamics and Tissue Micro-architecture in Immune Function"
National Institute of Allergy and Infectious Diseases, Bethesda (US) / 12.03.2013

Falk Nimmerjahn
"Understanding and modulating the activity of IgG in vivo"
University of Erlangen-Nuremberg, Erlangen (DE) / 11.04.2013

Sara Rankin
"Trafficking of progenitor cells in vivo"
Imperial College London, London (UK) / 22.05.2013

John Mascola
"The Development of HIV-1 Neutralizing Antibodies"
National Institutes of Health, Bethesda (US) / 12.06.2013

James P. Allison
"Immune Checkpoint Blockade in Cancer Therapy: New Insights and Opportunities"
Memorial Sloan-Kettering Cancer Center, New York (US) / 21.08.2013

PhD Lecture Course 2013 - 2014

Andrea Cerutti
"Innate signaling networks in splenic and intestinal immune responses"
Hospital del Mar Medical Research Institute, Barcelona (ES) / 06.11.2013

Ana Cuervo
"Selective autophagy: fighting aging one protein at a time"
Albert Einstein College of Medicine, New York (US) / 19.12.2013

Jim Di Santo
"Transcriptional Regulation of Innate Lymphocyte Differentiation"
Pasteur Institute, Paris (FR) / 18.02.2014

Yasmine Belkaid
"Role of the microbiota in local and systemic control of immunity"
National Institute of Allergy and Infectious Diseases, Bethesda (US) / 18.03.2014

Leo James
"Intracellular antibodies - in the wrong place, at the right time"
Medical Research Council, Laboratory of Molecular Biology, Cambridge (UK) / 28.03.2014

Thomas Winkler
"B cell and T cell immunity in MCMV reactivation after allogeneic bone marrow transplantation"
University of Erlangen-Nuremberg, Erlangen (DE) / 24.04.2014

Stephen T. Smale
"Transcriptional cascades associated with innate immunity and inflammation"
University of California, Los Angeles (US) / 27.05.2014

Ed Palmer
"Strong-self / Weak-self: How T lineage cells calculate antigen affinity and avoid autoimmunity"
University Hospital Basel, Basel (CH) / 10.06.2014

Jacques Neefjes
"The systems biology of MHC class II antigen presentation and application in DC biology"
Netherlands Cancer Institute, Amsterdam (NL) / 20.06.2014

Seminar Programme 2013

Maria Zambon
"SARS again? : Novel coronavirus 2012"
Health Protection Agency, London (UK) / 10.01.2013

Manfredi Di San Germano
"Introduction to BGI and its current research directions"
BGI Europe, Copenhagen (DK) / 10.01.2013

Rocco Falchetto
"Analytical Sciences in Drug Discovery"
NIBR Analytical Sciences, Novartis Basel, Basel (CH) / 01.02.2013

Laura Solforosi
"Identifying key components of the PrPC-PrPSc interactions and replicative interface"
Institute of Microbiology and Virology, Università Vita-Salute San Raffaele, Milan (IT) / 04.03.2013

Ubaldo E. Martinez Outschoorn
"Two Compartment Tumor Metabolism"
Departments of Medical Oncology and of Stem Cell Biology and Regenerative Medicine, Thomas Jefferson University Hospital, Philadelphia (US) / 08.03.2013

Karl Schenck
"All in the family? Streptococcus mitis and Streptococcus pneumoniae"
Department of Oral Biology, University of Oslo, Oslo (NO) / 18.03.2013

Peter Lüthy
"Basic principles and application of Bacillus thuringiensis"
Institute of Microbiology, Department of Biology, ETH Zürich, Zürich (CH) / 12.04.2013

Arnaud Marchant
"B cell response to primary CMV infection"
Institute for Medical Immunology, Université Libre de Bruxelles, Brussels (BE) / 14.05.2013

Eelco van Anken
"Endoplasmic Reticulum Stress Signaling Centers in Sight"
Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milan (IT) / 24.05.2013

Federica Facciotti
"IL-10 producing T cell subsets with opposing functions in B cell help"
Istituto Nazionale di Genetica Molecolare (INGM), Milan (IT) / 11.06.2013

Aurélien Rizk
"An ImageJ/Fiji plugin for segmenting and quantifying sub-cellular structures in fluorescence microscopy images"
Laboratory of Biomolecular Research, Paul Scherrer Institut, Villigen (CH) / 18.06.2013

Alan Aderem
"A Systems approach to dissecting Immunity"
Seattle Biomedical Research Institute, Seattle (US) / 26.06.2013

Roberto Cattaneo
"Measles virus r/evolution: from pathogen to cancer therapeutic"
Mayo Graduate School, Mayo Clinic, Rochester (US) / 27.06.2013

Gideon Berke
"Reflections on CTL action in Health and Disease"
Department of Immunology, Weizmann Institute of Science, Rehovot (IL) / 19.08.2013

Padmanee Sharma
"Immune Monitoring on Pre-Surgical Clinical Trials with Anti-CTLA-4"
The University of Texas MD Anderson Cancer Center, Houston (US) / 21.08.2013

Dietmar Zaiss
"Unexpected role of the EGF-R in the immune system"
Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, University of Utrecht, Utrecht (NL) / 28.08.2013

Michel Letzelter
"New technologies to accelerate the development of Protein Therapeutics, Antibodies and Vaccines"
PerkinElmer Switzerland (CH) / 28.08.2013

Connie Duong
"Generating Stronger and Smarter T cells for Adoptive Immunotherapy"
Immunology, Immunopathology and Immunotherapy Research Group, Department of Medicine and Biology,
Immune Innovation Laboratory, Division of Cancer Immunology, Peter MacCallum Cancer Centre, East Melbourne (AU) / 11.09.2013

Mario P. Tschan
"Deregulated Autophagy Gene Expression Contributes to the Differentiation Block in Acute Myeloid Leukemia"
Institute of Pathology, University of Bern, Bern (CH) / 20.09.2013

Kathrin Pieper
"A common SNP in BAFF-R impairs receptor multimerization and contributes to disease susceptibility"
Center of Chronic Immunodeficiency, University Medical Center Freiburg, Freiburg (DE) / 20.09.2013

Emilio Hirsch
"PI3K class II Alpha in development and cancer"
Department of Genetics, Biology and Biochemistry, University of Torino, Torino (IT) / 03.10.2013

Annalies Zinkernagel
"It takes two to tango: host streptococcal interactions"
Division on Infectious Diseases and Hospital Epidemiology, University Hospital Zürich, Zürich (CH) / 04.10.2013

Paolo Paganetti
"Approaches for Disease Modifying Therapies of Neurodegenerative Disorders"
Experimental Research Laboratory, Neurocenter of Southern Switzerland (NSI), Bellinzona (CH) / 30.10.2013

Claudia Daubenberger
"Development of influenza virosome-based synthetic malaria vaccines"
Swiss Tropical and Public Health Institute (Swiss TPH), Basel (CH) / 05.11.2013

Nicolas Fasel
"Metastatic leishmaniasis: when the host pays the toll"
Center for Immunity and Infection Lausanne, University of Lausanne, Epalinges (CH) / 12.11.2013

Lars Klareskog
"Genes, environment and adaptive immunity in rheumatoid arthritis"
Department of Medicine (Solna), Karolinska University Hospital, Stockholm (SE) / 13.12.2013

Fernando Macian-Juan
"Positive and negative regulation of T cell activation: autophagy and epigenetics at work"
Albert Einstein College of Medicine, New York (US) / 18.12.2013

Seminar Programme 2014

Alain R. Townsend
"Active and Passive Immunity to Influenza"
Weatherall Institute of Molecular Medicine, University of Oxford, Oxford (UK) / 19.02.2014

Manfred Kopf
"Lipid oxidation and T cell responses"
Institute for Molecular Health Sciences, ETH Zürich, Zürich (CH) / 20.02.2014

Stephan Urban
"Entry and entry inhibition of Hepatitis B Virus: The role of NTCP as a bone fide HBV receptor"
Division of Molecular Virology, Department of Infectious Diseases, University Hospital Heidelberg, Heidelberg (DE) / 03.03.2014

Nicolas Delaleu
"Advancing systematic extraction of biological meaning from 'omics' datasets:
Mapping emerging experimental Sjögren's syndrome and responses to treatment"
Broegelmann Research Laboratory, Department of Clinical Science, University of Bergen, Bergen (NO) / 01.04.2014

Esther Ketelaars
"Characterisation of anti-SOIV antibodies and analysis of influenza escape mutants"
Department of Biology, ETH Zürich, Zürich (CH) / 08.04.2014

Jacopo Sgrignani
"Molecular modeling of pharmaceutically relevant enzymes"
Istituto di Chimica del Riconoscimento Molecolare (ICRM), National Research Council (CNR), Milan (IT) / 03.06.2014

Jeffrey Rathmell
"Metabolism of CD4 T cell subsets and inflammatory disease"
Department of Pharmacology & Cancer Biology, Duke University School of Medicine, Durham (US) / 13.06.2014

Peter D. Kwong
"Structure and vaccine design with broadly neutralizing antibody MPE8"
Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda (US) / 16.06.2014

Christoph Schlapbach
"IL-9 producing T helper cells: Are they a T helper cell subset?"
Department of Dermatology, Inselspital / Bern University Hospital, Bern (CH) / 07.07.2014

Vincenzo Barnaba
"Epi-autoimmunity in viral and autoimmune diseases"
Dipartimento di Medicina Interna e Specialità Mediche, Università degli Studi di Roma "La Sapienza", Rome (IT) / 25.09.2014

Peter Bull
"Exploring the role of Infected erythrocyte surface antigens as targets of naturally acquired immunity to malaria"
KEMRI-Wellcome Trust Programme, Kilifi (KE) / 07.10.2014

Torsten Ochsenreiter
"Scientific Integrity"
Institute of Cell Biology, University of Bern, Bern (CH) / 29.10.2014

Torsten Ochsenreiter
"A bonding experience: The composition, hierarchy and dynamics of the mitochondrial genome segregation complex in trypanosomes"
Institute of Cell Biology, University of Bern, Bern (CH) / 29.10.2014

Roberto Baccala
"Type I Interferons in autoimmunity and viral immunopathology"
Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla (US) / 03.11.2014

Thomas M. Bauer
"A Novel way of Multiple Cytokine detection: two dimensional readout in a three dimensional assay"
AYOXXA Biosystems GmbH, Cologne (DE) / 06.11.2014

Francisco J. Quintana
"Regulation of CNS inflammation"
Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston (US) / 07.11.2014

PEOPLE & FINANCES

SECTION 6

INDEX SECTION 6 – PEOPLE & FINANCES

IRB PERSONNEL

ADMINISTRATION

Director
Antonio Lanzavecchia

Vice Director
Mariagrazia Uguccioni

Administrative Director
Guido Turati (Fidinam)

Administration
Fosca Bognuda
Giada Bosia
Denise Dos Santos Neves
Jelena Markovic Jovic
Adelle Parsons
Jessica Roberti Zanellato
Fabiano Wildi

Grant officer
Maryse Letiembre

Veterinarian
Melania Osto

RESEARCH STAFF

Group Leaders
Santiago F. González
Fabio Grassi
Antonio Lanzavecchia
Maurizio Molinari
Silvia Monticelli
Federica Sallusto
Marcus Thelen
Mariagrazia Uguccioni
Luca Varani

Associate Member
Andrea Cavalli

Researchers
Martina Beltramello
Riccardo Bernasconi
Costanza Casiraghi
Valentina Cecchinato
Nikolaos Chatziandreou
Davide Corti
Darius Ekonomiuk
Carmela Galli
Roger Geiger
Jérémie Goldstein
Gabor Gyülvészi
Anna Kabanova
Daniela Latorre
Elisabetta Loggi
Roberta Marzi
Samuele Notarbartolo
Simon Olsson
Mattia Pedotti
Kathrin Pieper
Debora Pinna
Michele Proietti
Lorenzo Raeli
Luca Simonelli
Jordi Sintes
Jacopo Sgrignani
Tatiana Soldà
Sylvia Thelen

Students
Dominik Aschenbrenner
Simona Baldassari
Marco Bardelli
Camilla Basso
Simone Becattini
Thimothy Bergmann
Giorgia Brambilla Pisoni
Elena Brenna
Tess Brodie
Anna Casati
Sabrina Casella
Antonino Cassotta
Flavio Cueni
Gianluca D'Agostino
Corinne De Gregorio
Lorenzo Dehò
Caterina Elisa Faliti
Yagmur Farsakoglu
Ilaria Fregno
Alexander Frühwirth
Fiorenza Fumagalli
Dario Fuschillo
Valentina Gilardi
Louise Humpert
Daniela Iannotta
Daniela Impellizzieri
Esther Ketelaars
Alessia Landi
Cristina Leoni
Elsa Livoti
Matteo Mauri
Federico Mele
Jessica Merulla
Sara Montagner
Francesco Müller
Sara Natali
Julia Noack
Miguel Palomino
Leontios Pappas
Lisa Perruzza
Luca Piccoli
Dora Pinto
Diego Pizzagalli
Silvia Preite
Viola Puddinu
Tanja Rezzonico Jost
Egle Radice
Sara Ravasio
Andrea Romagnani
Elsa Rottoli
Karolin Rommel
Joshua Hoong Yu Tan

Lucia Vincenzetti
Florian Wimmers
Tobias Wolf
Tomasz Wypych
Silvia Zanaga

Technicians
Maria Gabriela Danelon
Elisa Fasana
Blanca Fernandez-Rodriguez
Mathilde Foglierini Perez
Isabella Giacchetto-Sasselli
Sandra Jovic
Andrea Minola
Luana Perlini
Chiara Silacci Fregni

Imaging Facility
Rocco D'Antuono
David Jarrossay
Erica Montani

GEPP Facility
Laurent Perez
Jessica Marcandalli
Lina Maciariello

Animal House Facility
Ghassan Bahnan
Emanuele Cavadini
Andrea D'Ercole
Toma Kobkyn
Enrica Mira Catò

Support Staff
Ronnie Baccalà
Mauro Pasteris
Beatrice Pasteris

IT Support
Andrea Dellavia (TI-EDU)
Ivano Di Remigio (TI-EDU)
TI-EDU Team

FOUNDATION COUNCIL

Gabriele Gendotti, President *
Felice Zanetti, Vice-President
Paolo Agustoni *
Franco Cavalli
Hans Hengartner
Carlo Maggini
Piero Martinoli *
Dario Neri
Giorgio Noseda*
Jean-Claude Piffaretti
Sandro Rusconi
Alberto Togni *

* Member of the Executive Committee

SCIENTIFIC ADVISORY BOARD

Adriano Aguzzi
University Hospital Zurich (CH)
Stefan Kaufmann
Max Planck Institute for Infection Biology Berlin (DE)
Alberto Mantovani
University of Milan (IT)
Cesare Montecucco
University of Padova (IT)
Anne O'Garra
National Institute for Medical Research London (UK)

DONORS

Core Funding

The Helmut Horten Foundation
The City of Bellinzona
The Canton of Ticino
The Swiss Confederation
Gustav & Ruth Jacob Foundation

Major Donors

Signora Alessandra
Banca della Svizzera Italiana
COMEL Foundation
ESOF Foundation
Fondazione ADIUVARE
Fondazione del Ceresio
Fondazione Coromandel
Fondazione Fidinam
Fondazione Gelu
Fondazione Henry Krenter
Fondazione Sievers
Fondazione per lo studio
delle malattie neurodegenerative
Monique Fulcieri
The Gabriele Charitable Trust
Augusto Gallera
Heinrich & Myriam Gebert
GGG Foundation
Dorina Guidotti
Gurta SA
Gurten & Jauch
Silvio Leoni
Sergio Monti
Pina Petroli SA
Jost Reinhold
Ricerca Svizzera contro il Cancro
Fondazione San Salvatore
Silva Casa Foundation
Stelinvest
Tito Tettamanti
Meynarda & Jantje Van Terwisga
Hans Wyder

Friends of the Institute

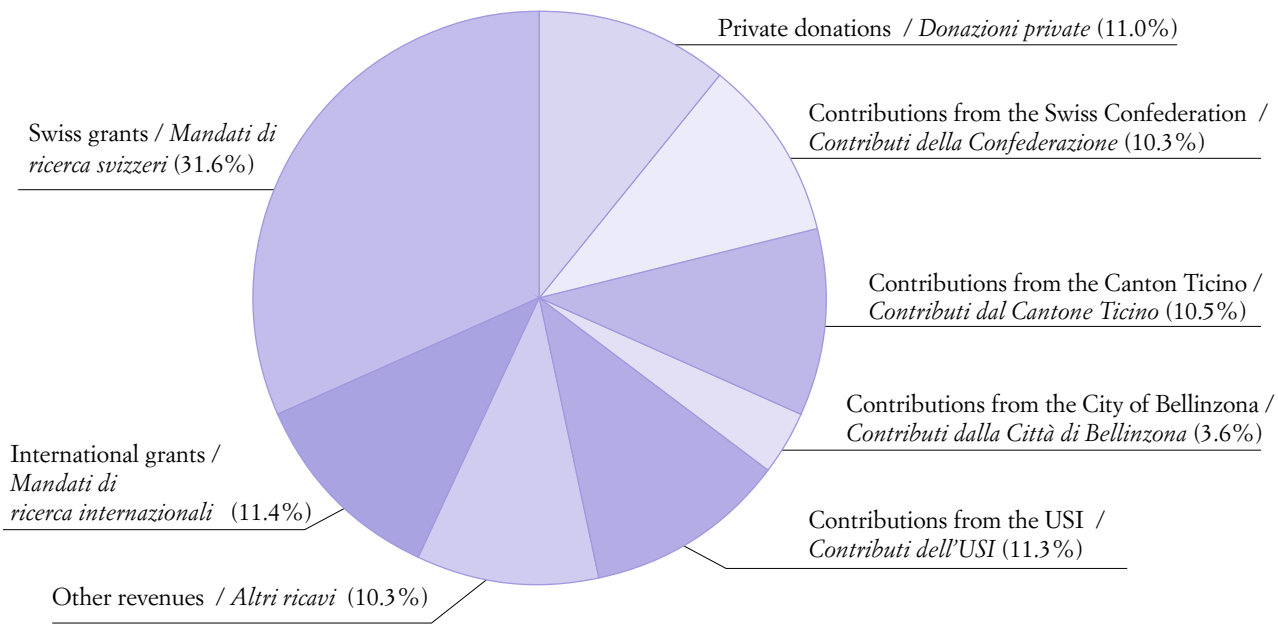
Fabio Abate
G Ambrosini
Giuseppe Antonioli
Marco Agustoni
Marco G Berini
Giancarlo Berther
Carlo Bertinelli
Armando Boneff
Marisa Bonzanigo
Luisella Bonzanigo-Burini
Carlo Borradori
Massimo Brandini
Livio Casellini
Simona Cetti
Carlo e Antonella Chicherio
Ekaterina Chigrinova
Andrea Crespi
Del Biaggio-Bianchi
Michela Delcò Petralli
Giacomo Ghezzi
Giovanni Ghezzi
Dario Ghisletta
Erminio Giudici
Iva Jauch
Eliane Lotrecchiano
Carlo E Michelotti
Davide Molo
Francesca R Molo
Rodolfo Molo
Rasini & Sigon
Iginio Rezzonico
Aldo Robustelli
Lauro e Miriam Rotanza
Rosalia Sansossio-Cippà
Loredana Schlegel-Pacciorini
Augusto Solari
Corrado Solcà
Taurus Asset Management SA
Fausto Tognacca
Fernanda Tognacca
Teodolinda Tognacca

Financial Data 2013-2014 (in Swiss Francs) / *Dati finanziari 2013-2014 (in Franchi svizzeri)*

Gli esercizi 2013 e 2014 hanno visto una forte evoluzione del budget della Fondazione. L'affiliazione all'USI con i relativi nuovi contributi e alcuni finanziamenti straordinari hanno permesso di effettuare importanti investimenti in nuove attrezzature per 3,8 milioni CHF nel biennio 2013-2014, oltre a costituire due Fondi strategici per il futuro dell'IRB per 1,9 milioni CHF: un fondo dotato di 0,5 milioni CHF con lo scopo di finanziare la fase di progettazione del nuovo stabile, un altro con 1,4 milioni CHF con lo scopo di contribuire a garantire il finanziamento a lungo termine dell'attività di ricerca, attività che in entrambi gli anni ha superato gli 8 milioni di CHF. Gli importanti finanziamenti ottenuti con i progetti di ricerca hanno permesso di finanziare, nel 2013 il 49,3 % e nel 2014 il 43,6 %, dell'intero budget della Fondazione.

Thanks to the USI affiliation, which brought new contributions and some special funding, it was possible to make major investments in new equipment for CHF 3.8 million in the 2013-2014 period, and to constitute two strategic funds for the future new IRB headquarters for CHF 1.9 million. One strategic fund (CHF 0.5 million) was constituted to finance the design phase of the new building, while another one (CHF 1.4 million) was constituted to help ensure the long-term funding of the research activities that in both years exceeded CHF 8 million. The outstanding grants obtained by the IRB researchers represented 49.3% in 2013 and 43.6% in 2014 of the entire budget of the Foundation.

Funding by source 2014 / *Contributi per fonte 2014*



Balance Sheet as 31 of December 2013 and as 31 of December 2014 (In Swiss Francs)
Bilancio al 31 dicembre 2013 ed al 31 dicembre 2014 (in Franchi Svizzeri)

ASSETS / *ATTIVO*		31.12.2014	31.12.2013
1.	Liquidity / *Liquidità*	12'317'997	12'207'162
2.	Receivables / *Crediti*	864'531	1'037'433
3.	Temporary Receivables / *Transitori attivi*	3'472'350	1'516'938
	Current Assets / *Attivo circolante*	16'654'878	14'761'533
4.	Participations / *Partecipazioni*	12'500	12'500
5.	Financial investments / *Investimenti finanziari*	2'000'000	2'000'000
6.	Buildings / *Immobili*	2'812'440	3'767'440
7.	Furnishing & Equipment / *Attrezzature*	1	40'000
	Fixed Assets / *Attivo fisso*	4'824'941	5'819'940
Total Assets / *Totale attivo*		21'479'819	20'581'473

LIABILITIES / *PASSIVO*		31.12.2014	31.12.2013
1.	Payables for goods and services / *Debiti per forniture e prestazioni*	660'096	692'172
2.	Accruals / *Accantonamenti e transitori passivi*	586'365	397'865
3.	Funds for Research Projects / *Fondi progetti di ricerca*	3'616'813	5'093'950
4.	Funds for Laboratories / *Fondi dei laboratori*	2'779'215	2'251'302
5.	Various Funds / *Fondi diversi*	2'067'863	1'848'770
	Current Liabilities / *Capitale estraneo a breve termine*	9'710'352	10'284'059
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*	7'497'414	7'390'363
8.	Strategic Fund / *Fondo Strategico*	1'400'000	0
9.	Annual Result / *Risultato d'esercizio*	72'053	107'051
	Equity of the Foundation / *Capitale della Fondazione*	8'969'467	7'497'414
Total Liabilities / *Totale passivo*		21'479'819	20'581'473

Profit and Loss Account for the years 2013 and 2014 (In Swiss Francs) / *Conti economici esercizi 2013 e 2014 (in Franchi svizzeri)*

COSTS / *COSTI*		2014	2013
1.	Personnel Costs / *Costi del personale*	7'842'993	7'639'255
2.	Consumables / *Fabbisogno medico*	2'271'482	2'200'775
3.	Maintenance of Buildings and Equipment / *Manutenzione immobili e attrezzature*	867'513	862'766
4.	Investments / *Investimenti*	1'561'993	2'233'055
5.	Amortizations / *Ammortamenti*	1'000'412	1'004'681
6.	Rent and Related Costs / *Affiti e altri costi dei locali*	1'321'149	1'324'961
7.	Administrative Costs and Various / *Costi generali amministrativi e diversi*	893'598	960'448
8.	Travels, Congresses and Guests / *Trasferte, congressi, viaggi e ospiti*	300'037	300'746
9.	Constitution of Funds / *Costituzione fondi*	1'500'000	400'000
10.	Various Costs for Research / *Altri costi di ricerca*	988'177	715'847
Total Costs / *Totale costi*		18'547'355	17'642'534

REVENUES / *RICAVI*		2014	2013
1.	Contributions from the Confederation / *Contributi Confederazione*	1'950'000	1'800'000
2.	Contribution from the Canton Ticino / *Contributi Canton Ticino*	1'978'000	2'022'000
3.	Contribution from the City of Bellinzona / *Contributi Città di Bellinzona*	681'000	680'860
4.	Contributions from USI / *Contributi USI*	2'124'900	818'279
5.	Contributions from the Helmut Horten Foundation / *Contributi Fondazione Helmut Horten*	1'500'000	1'768'000
6.	Other Contributions / *Altri Contributi*	1'104'954	1'155'140
7.	Research Projects / *Progetti di ricerca*	8'116'352	8'512'517
8.	Other Revenues / *Altri ricavi*	1'164'201	992'789
Total Revenues / *Totale ricavi*		18'619'408	17'749'585

ANNUAL RESULT / RISULTATO D'ESERCIZIO	**72'053**	**107'051**

PUBLICATIONS
SECTION 7

INDEX SECTION 7 – PUBLICATIONS

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

Peer Reviewed Publications from January 2013

333. **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.

334. **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.

335. **HMGB1 and leukocyte migration during trauma and sterile inflammation.**
Venereau, E., M. Schiraldi, M. Uguccioni and M. E. Bianchi
Mol Immunol. 2013; 55:76-82.

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; 62:1665-1675.

337. **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.

338. **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.

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. **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.

343. **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.

344. **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
Immunity. 2013; 38:596-605.

345. **OMIP-018: Chemokine receptor expression on human T helper cells.**
Brodie, T., E. Brenna and F. Sallusto
Cytometry A. 2013; 83:530-532.

346. **Broadly neutralizing antiviral antibodies.**
Corti, D. and A. Lanzavecchia
Annu Rev Immunol. 2013; 31:705-742.

347. **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; 43:1651-1658.

348. **Specificity and regulation of the endoplasmic reticulum-associated degradation machinery.**
Merulla, J., E. Fasana, T. Solda and M. Molinari
Traffic. 2013; 14:767-777.

349. **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
PLoS One. 2013; 8:e59863.

350. **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; 121:4110-4114.

351. **Immune response: steroids drive dendritic cells.**
Jarrossay, D. and M. Thelen
Nat Immunol. 2013; 14:424-426.

352. **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.

353. **Anti-HIV IgA isotypes: differential virion capture and inhibition of transcytosis are linked to prevention of mucosal R5 SHIV transmission.**
Watkins, J. D., A. M. Sholukh, M. M. Mukhtar, N. B. Siddappa, S. K. Lakhashe, M. Kim, E. L. Reinherz, S. Gupta, D. N. Forthal, Q. J. Sattentau, F. Villinger, D. Corti, R. M. Ruprecht and C. P. G. for the
AIDS. 2013; 27:F13-F20.

354. **Dependence of immunoglobulin class switch recombination in B cells on vesicular release of ATP and CD73 ectonucleotidase activity.**
Schena, F., S. Volpi, C. E. Faliti, F. Penco, S. Santi, M. Proietti, U. Schenk, G. Damonte, A. Salis, M. Bellotti, F. Fais, C. Tenca, M. Gattorno, H. Eibel, M. Rizzi, K. Warnatz, M. Idzko,

C. K. Ayata, M. Rakhmanov, T. Galli, A. Martini, M. Canossa, F. Grassi and E. Traggiai
Cell Rep. 2013; 3:1824-1831.

355. **The N276 Glycosylation Site Is Required for HIV-1 Neutralization by the CD4 Binding Site Specific HJ16 Monoclonal Antibody.**
Balla-Jhagjhoorsingh, S. S., D. Corti, L. Heyndrickx, E. Willems, K. Vereecken, D. Davis and G. Vanham
PLoS One. 2013; 8:e68863.

356. **UDP-glucose:glycoprotein glucosyltransferase (UGGT1) promotes substrate solubility in the endoplasmic reticulum.**
Ferris, S. P., N. S. Jaber, M. Molinari, P. Arvan and R. J. Kaufman
Mol Biol Cell. 2013; 24:2597-2608.

357. **Transgenic expression of beta1 antibody in brain neurons impairs age-dependent amyloid deposition in APP23 mice.**
Paganetti, P., J. Reichwald, D. Bleckmann, D. Abramowski, D. Ammaturo, C. Barske, S. Danner, M. Molinari, M. Muller, S. Papin, S. Rabe, P. Schmid and M. Staufenbiel
Neurobiol Aging. 2013; 34:2866-2878.

358. **Short-term memory of danger signals and environmental stimuli in immune cells.**
Monticelli, S. and G. Natoli
Nat Immunol. 2013; 14:777-784.

359. **Emergence of New Pandemic GII.4 Sydney Norovirus Strain Correlates With Escape From Herd Immunity.**
Debbink, K., L. C. Lindesmith, E. F. Donaldson, V. Costantini, M. Beltramello, D. Corti, J. Swanstrom, A. Lanzavecchia, J. Vinje and R. S. Baric
J Infect Dis. 2013; 208:1877-1887.

360. **Cross-neutralization of four paramyxoviruses by a human monoclonal antibody.**
Corti, D., S. Bianchi, F. Vanzetta, A. Minola, L. Perez, G. Agatic, B. Guarino, C. Silacci, J. Marcandalli, B. J. Marsland, A. Piralla, E. Percivalle, F. Sallusto, F. Baldanti and A. Lanzavecchia
Nature. 2013; 501:439-443.

361. **DCs: a dual bridge to protective immunity.**
Sallusto, F.
Nat Immunol. 2013; 14:890-891.

362. **Expression of the Chemokine Receptor CXCR7 in CXCR4-Expressing Human 143B Osteosarcoma Cells Enhances Lung Metastasis of Intratibial Xenografts in SCID Mice.**
Brennecke, P., M. J. Arlt, R. Muff, C. Campanile, A. Gvozdenovic, K. Husmann, N. Holzwarth, E. Cameroni, F. Ehrensperger, M. Thelen, W. Born and B. Fuchs
PLoS One. 2013; 8:e74045.

363. **Highly Significant Antiviral Activity of HIV-1 LTR-Specific Tre-Recombinase in Humanized Mice.**
Hauber, I., H. Hofmann-Sieber, J. Chemnitz, D. Dubrau, J. Chusainow, R. Stucka, P. Hartjen, A. Schambach, P. Ziegler, K. Hackmann, E. Schrock, U. Schumacher, C. Lindner, A. Grundhoff, C. Baum, M. G. Manz, F. Buchholz and J. Hauber
PLoS Pathog. 2013; 9:e1003587.

364. **Receptor-based high-throughput screening and identification of estrogens in dietary supplements using bioaffinity liquid-chromatography ion mobility mass spectrometry.**
Aqai, P., N. G. Blesa, H. Major, M. Pedotti, L. Varani, V. E. Ferrero, W. Haasnoot and M. W. Nielen
Anal Bioanal Chem. 2013; 405:9427-9436.

365. **Continuous monitoring of Naproxen by a cytochrome P450-based electrochemical sensor.**
Baj-Rossi, C., T. Rezzonico Jost, A. Cavallini, F. Grassi, G. De Micheli and S. Carrara
Biosens Bioelectron. 2014; 53:283-287.

366. **Opening Study on the Development of a New Biosensor for Metal Toxicity Based on Pseudomonas fluorescens Pyoverdine.**
Chiadò, A., L. Varani, F. Bosco and L. Marmo
Biosensors in Environmental Studies. 2013; 4:385-399.

367. **The who's who of T-cell differentiation: Human memory T-cell subsets.**
Mahnke, Y. D., T. M. Brodie, F. Sallusto, M. Roederer and E. Lugli
Eur J Immunol. 2013; 43:2797-2809.

368. **Replica-Averaged Metadynamics.**
Camilloni, C., A. Cavalli and M. Vendruscolo
J. Chem. Theory Comput. . 2013; 12:5610-5617.

369. **International Union of Pharmacology. LXXXIX. Update on the Extended Family of Chemokine Receptors and Introducing a New Nomenclature for Atypical Chemokine Receptors.**
Bachelerie, F., A. Ben-Baruch, A. M. Burkhardt, C. Combadiere, J. M. Farber, G. J. Graham, R. Horuk, A. H. Sparre-Ulrich, M. Locati, A. D. Luster, A. Mantovani, K. Matsushima, P. M. Murphy, R. Nibbs, H. Nomiyama, C. A. Power, A. E. Proudfoot, M. M. Rosenkilde, A. Rot, S. Sozzani, M. Thelen, O. Yoshie and A. Zlotnik
Pharmacol Rev. 2014; 66:1-79.

370. **MicroRNAs in T helper cell differentiation and plasticity.**
Monticelli, S.
Semin Immunol. 2013; 25:291-298.

371. **The many faces of CD4 T cells: Roles in immunity and disease.**
Sallusto, F. and S. Monticelli
Semin Immunol. 2013; 25:249-251.

372. **CXCR7 influences the migration of B cells during maturation.**
Humpert, M. L., D. Pinto, D. Jarrossay and M. Thelen
Eur J Immunol. 2014; 44:694-705.

373. **The Neonatal Fc Receptor (FcRn) Enhances Human Immunodeficiency Virus Type 1 (HIV-1) Transcytosis across Epithelial Cells.**
Gupta, S., J. S. Gach, J. C. Becerra, T. B. Phan, J. Pudney, Z. Moldoveanu, S. B. Joseph, G. Landucci, M. J. Supnet, L. H. Ping, D. Corti, B. Moldt, Z. Hel, A. Lanzavecchia, R. M. Ruprecht, D. R. Burton, J. Mestecky, D. J. Anderson and D. N. Forthal
PLoS Pathog. 2013; 9:e1003776.

374. **A conformational ensemble derived using NMR methyl chemical shifts reveals a mechanical clamping transition that gates the binding of the HU protein to DNA.**
Kannan, A., C. Camilloni, A. B. Sahakyan, A. Cavalli and M. Vendruscolo
J Am Chem Soc. 2014; 136:2204-2207.

375. **CXCR4 antibody treatment suppresses metastatic spread to the lung of intratibial human osteosarcoma xenografts in mice.**
Brennecke, P., M. J. Arlt, C. Campanile, K. Husmann, A. Gvozdenovic, T. Apuzzo, M. Thelen, W. Born and B. Fuchs
Clin Exp Metastasis. 2014; 31:339-349.

376. **Determination of the individual roles of the linker residues in the interdomain motions of calmodulin using NMR chemical shifts.**
Kukic, P., C. Camilloni, A. Cavalli and M. Vendruscolo
J Mol Biol. 2014; 426:1826-1838.

377. **Macrophage activation: glancing into diversity.**
Natoli, G. and S. Monticelli
Immunity. 2014; 40:175-177.

378. **New nomenclature for atypical chemokine receptors.**
Bachelerie, F., G. J. Graham, M. Locati, A. Mantovani, P. M. Murphy, R. Nibbs, A. Rot, S. Sozzani and M. Thelen
Nat Immunol. 2014; 15:207-208.

379. **Within-Host Evolution Results in Antigenically Distinct GII.4 Noroviruses.**
Debbink, K., L. C. Lindesmith, M. T. Ferris, J. Swanstrom, M. Beltramello, D. Corti, A. Lanzavecchia and R. S. Baric
J Virol. 2014; 88:7244-7255.

380. **ALMOST: An all atom molecular simulation toolkit for protein structure determination.**
Fu, B., A. B. Sahakyan, C. Camilloni, G. G. Tartaglia, E. Paci, A. Caflisch, M. Vendruscolo and A. Cavalli
J Comput Chem. 2014; 35:1101-1105.

381. **MicroRNAs in hematopoietic development.**
Montagner, S., L. Deho and S. Monticelli
BMC Immunol. 2014; 15:14.

382. **CXCL11-dependent induction of FOXP3-negative regulatory T cells suppresses autoimmune encephalomyelitis.**
Zohar, Y., G. Wildbaum, R. Novak, A. L. Salzman, M. Thelen, R. Alon, Y. Barsheshet, C. L. Karp and N. Karin
J Clin Invest. 2014; 124:2009-2022.

383. **CXCR7 prevents excessive CXCL12-mediated downregulation of CXCR4 in migrating cortical interneurons.**
Abe, P., W. Mueller, D. Schutz, F. Mackay, M. Thelen, P. Zhang and R. Stumm
Development. 2014; 141:1857-1863.

384. **Particle Conformation Regulates Antibody Access to a Conserved GII.4 Norovirus Blockade Epitope.**
Lindesmith, L. C., E. F. Donaldson, M. Beltramello, S. Pintus, D. Corti, J. Swanstrom, K. Debbink, T. A. Jones, A. Lanzavecchia and R. S. Baric
J Virol. 2014; 88:8826-8842.

385. **A probabilistic approach to the determination of native state ensembles of proteins.**
Olsson, S., B. R. Vögeli, A. Cavalli, W. Boomsma, J. Ferkinghoff-Borg, K. Lindorff-Larsen and T. Hamelryck
J. Chem. Theory Comput. 2014; 10:3484-3491.

386. **How viruses hijack the ERAD tuning machinery.**
Noack, J., R. Bernasconi and M. Molinari
J Virol. 2014; 88:10272-10275.

387. **Innate immune cells express IL-17A/F in acute generalized exanthematous pustulosis and generalized pustular psoriasis.**
Kakeda, M., C. Schlapbach, G. Danelon, M. M. Tang, V. Cecchinato, N. Yawalkar and M. Uguccioni
Arch Dermatol Res. 2014; 306:933-938.

388. **Proteome-wide analysis of HIV-specific naive and memory CD4+ T cells in unexposed blood donors.**
Campion, S. L., T. M. Brodie, W. Fischer, B. T. Korber, A. Rossetti, N. Goonetilleke, A. J. McMichael and F. Sallusto
J Exp Med. 2014; 211:1273-1280.

389. **Two functionally distinct subsets of mast cells discriminated By IL-2-independent CD25 activities.**
Deho, L., C. Leoni, T. M. Brodie, S. Montagner, M. De Simone, S. Polletti, I. Barozzi, G. Natoli and S. Monticelli
J Immunol. 2014; 193:2196-2206.

390. **Rational modification of estrogen receptor by combination of computational and experimental analysis.**
Ferrero, V. E., M. Pedotti, A. Chiado, L. Simonelli, L. Calzolai, T. Lettieri and L. Varani
PLoS One. 2014; 9:e102658.

391. **The Oral Commensal Streptococcus mitis Shows a Mixed Memory Th Cell Signature That Is Similar to and Cross-Reactive with Streptococcus pneumoniae.**
Engen, S. A., H. Valen Rukke, S. Becattini, D. Jarrossay, I. J. Blix, F. C. Petersen, F. Sallusto and K. Schenck
PLoS One. 2014; 9:e104306.

392. **Proteostasis: Bad news and good news from the endoplasmic reticulum.**
Noack, J., G. Brambilla Pisoni and M. Molinari
Swiss Med Wkly. 2014; 144:w14001.

393. **Tumour-infiltrating Gr-1+ myeloid cells antagonize senescence in cancer.**
Di Mitri, D., A. Toso, J. J. Chen, M. Sarti, S. Pinton, T. R. Jost, R. D'Antuono, E. Montani, R. Garcia-Escudero, I. Guccini, S. Da Silva-Alvarez, M. Collado, M. Eisenberger, Z. Zhang, C. Catapano, F. Grassi and A. Alimonti
Nature. 2014; 515:134-137.

394. **Rag Defects and Thymic Stroma: Lessons from Animal Models.**
Marrella, V., P. L. Poliani, L. D. Notarangelo, F. Grassi and A. Villa
Front Immunol. 2014; 5:407.

395. **Autoantibodies to neurofascin-186 and gliomedin in multifocal motor neuropathy.**
Notturno, F., T. Di Febo, N. Yuki, B. M. Fernandez Rodriguez, D. Corti, E. Nobile-Orazio, M. Carpo, A. De Lauretis and A. Uncini
J Neuroimmunol. 2014; 276:207-212.

396. **Enhancing chemotherapy efficacy in Pten-deficient prostate tumors by activating the senescence-associated antitumor immunity.**
Toso, A., A. Revandkar, D. Di Mitri, I. Guccini, M. Proietti, M. Sarti, S. Pinton, J. Zhang, M. Kalathur, G. Civenni, D. Jarrossay, E. Montani, C. Marini, R. Garcia-Escudero, E. Scanziani, F. Grassi, P. P. Pandolfi, C. V. Catapano and A. Alimonti
Cell Rep. 2014; 9:75-89.

397. **Rapid development of broadly influenza neutralizing antibodies through redundant mutations.**
Pappas, L., M. Foglierini, L. Piccoli, N. L. Kallewaard, F. Turrini, C. Silacci, B. Fernandez-Rodriguez, G. Agatic, I. Giacchetto-Sasselli, G. Pellicciotta, F. Sallusto, Q. Zhu, E. Vicenzi, D. Corti and A. Lanzavecchia
Nature. 2014; 516:418-422.

398. **FRT - Fondation Rene Touraine: Pour La Dermatologie.**
Sakaguchi, S., F. Sallusto, T. Gebhardt, G. Stingl, R. A. Clark, L. F. Santamaria-Babi, E. Guttman-Yassky, G. Schuler and H. J. Koenen
Exp Dermatol. 2014; 23:772-785.

399. **Full fabrication and packaging of an implantable multi-panel device for monitoring of metabolites in small animals.**
Baj-Rossi, C., E. G. Kilinc, S. S. Ghoreishizadeh, D. Casarino, T. R. Jost, C. Dehollain, F. Grassi, L. Pastorino, G. De Micheli and S. Carrara
IEEE Trans Biomed Circuits Syst. 2014; 8:636-647.

400. **Protein trafficking: RESETting proteostasis.**
Noack, J. and M. Molinari
Nat Chem Biol. 2014; 10:881-882.

401. **LPS Nephropathy in Mice Is Ameliorated by IL-2 Independently of Regulatory T Cells Activity.**
Bertelli, R., A. Di Donato, M. Cioni, F. Grassi, M. Ikehata, A. Bonanni, M. P. Rastaldi and G. M. Ghiggeri
PLoS One. 2014; 9:e111285.

402. **ATP-Gated Ionotropic P2X7 Receptor Controls Follicular T Helper Cell Numbers in Peyer's Patches to Promote Host-Microbiota Mutualism.**
Proietti, M., V. Cornacchione, T. Rezzonico Jost, A. Romagnani, C. E. Faliti, L. Perruzza, R. Rigoni, E. Radaelli, F. Caprioli, S. Preziuso, B. Brannetti, M. Thelen, K. D. McCoy, E. Slack, E. Traggiai and F. Grassi
Immunity. 2014; 41:789-801.

403. **Interleukin-1 beta induces the expression and production of stem cell factor by epithelial cells: crucial involvement of the PI-3K/mTOR pathway and HIF-1 transcription complex.**
Wyszynski, R. W., B. F. Gibbs, L. Varani, D. Iannotta and V. V. Sumbayev
Cell Mol Immunol. 2014;

404. **Antibody-driven design of a human cytomegalovirus gHgLpUL128L subunit vaccine that selectively elicits potent neutralizing antibodies.**
Kabanova, A., L. Perez, D. Lilleri, J. Marcandalli, G. Agatic, S. Becattini, S. Preite, D. Fuschillo, E. Percivalle, F. Sallusto, G. Gerna, D. Corti and A. Lanzavecchia
Proc Natl Acad Sci U S A. 2014; 111:17965-17970.

405. **T cell immunity. Functional heterogeneity of human memory CD4(+) T cell clones primed by pathogens or vaccines.**
Becattini, S., D. Latorre, F. Mele, M. Foglierini, C. De Gregorio, A. Cassotta, B. Fernandez, S. Kelderman, T. N. Schumacher, D. Corti, A. Lanzavecchia and F. Sallusto
Science. 2015; 347:400-406.

406. **The immune receptor Tim-3 mediates activation of PI3 kinase/mTOR and HIF-1 pathways in human myeloid leukaemia cells.**
Prokhorov, A., B. F. Gibbs, M. Bardelli, L. Ruegg, E. Fasler-Kan, L. Varani and V. V. Sumbayev
Int J Biochem Cell Biol. 2014; 59C:11-20.

407. **Characterization of the Conformational Fluctuations in the Josephin Domain of Ataxin-3.**
Sanfelice, D., A. De Simone, A. Cavalli, S. Faggiano, M. Vendruscolo and A. Pastore
Biophys J. 2014; 107:2923-2931.

408. **N-linked sugar-regulated protein folding and quality control in the ER.**
Tannous, A., G. B. Pisoni, D. N. Hebert and M. Molinari
Semin Cell Dev Biol. 2014;

409. **Immunological consequences of intragenus conservation of Mycobacterium tuberculosis T-cell epitopes.**
Lindestam Arlehamn, C. S., S. Paul, F. Mele, C. Huang, J. A. Greenbaum, R. Vita, J. Sidney, B. Peters, F. Sallusto and A. Sette
Proc Natl Acad Sci U S A. 2015; 112:E147-155.

410. **Herpes simplex virus enhances chemokine function through modulation of receptor trafficking and oligomerization.**
Martinez-Martin, N., A. Viejo-Borbolla, R. Martin, S. Blanco, J. L. Benovic, M. Thelen and A. Alcami
Nat Commun. 2015; 6:6163.

411. **Insights into the coiled-coil organization of the Hendra virus phosphoprotein from combined biochemical and SAXS studies.**
Beltrandi, M., D. Blocquel, J. Erales, P. Barbier, S. Longhi and A. Cavalli
Virology. 2015; 477C:42-55.

412. **Reduced DNA methylation and hydroxymethylation in patients with systemic mastocytosis.**
Leoni, C., S. Montagner, L. Deho, R. D'Antuono, G. Matteis, A. V. Marzano, S. Merante, E. M. Orlandi, R. Zanotti and S. Monticelli
Eur J Haematol. 2015;

413. **A novel UGGT1 and p97-dependent checkpoint for native ectodomains with ionizable intramembrane residue.**
Merulla, J., T. Solda and M. Molinari
Mol Biol Cell. 2015

Book Chapters

Antibody-mediated immunity
Baumgarth, N., M. C. Carroll and S. González (2013).
Textbook of Influenza, 2nd Edition (eds R. G. Webster, A. S. Monto, T. J. Braciale and R. A. Lamb), John Wiley & Sons, Ltd, Oxford, UK.

IMPRESSUM
Istituto di Ricerca in Biomedicina
Institute for Research in Biomedicine
Via Vincenzo Vela 6 – 6500 Bellinzona
Tel. +41 91 820 0300
Fax +41 91 820 0305
www.irb.usi.ch info@irb.usi.ch

Layout: Tania Vanetti
Printing: Tipografia Torriani SA
Paper Cover: Normaset Puro 240 m^2
Pages: Normaset Puro 100 m^2
Running printing: 500