Università della Svizzera italiana

Institute for Research in Biomedicine

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Scientific Report
2015 - 2016

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This Scientific Report covers the 2015-2016 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

The Institute for Research in Biomedicine (IRB), a university-level institute based in Bellinzona and affiliated to the USI since 2010, showed again in 2016 that it was able to develop substantial basic research activity in an international context. Thanks to a leadership of international fame and to its high-level research activity, the IRB obtained important grants in several areas of competitive research and remained constantly present in a network of excellent collaborations. The Institute continues to distinguish itself for its unique arrangement that allows for the carrying out of scientific research at a high level with limited teaching activity. The core business of the IRB continues to be the study of defense mechanisms of the organism and the physiopathological basis of infectious, degenerative, inflammatory, and tumor diseases, with the goal of identifying new therapeutic strategies.

Also this year, the researchers of the IRB received prestigious prizes and recognitions on a national and international level. They have constantly appeared on the most eminent specialized journals with 53 publications with an impact factor (IF) of 10.5.
Important grants were awarded that contribute decisively to the funding of the scientific research of the Institute. In particular:

- Silvia Monticelli was awarded a grant from the National Center of Competence in Research (NCCR) 'RNA & Disease'. The NCCR 'RNA & Disease' is a research instrument of the Swiss National Science Foundation and is a coordinated and interdisciplinary program of research that aims to identify the mechanisms of diseases that derive from aberrant functions of RNA.
- Federica Sallusto is part of a consortium that won an Innovative Medicines Initiative (IMI) grant from the European Union. The project entitled "Development of immune tolerance therapies for the treatment of rheumatic diseases", RT-Cure, is the second project of this type obtained by researchers of the IRB.
- Mariagrazia Uguccioni is part of a consortium that won an international competition to develop new activities of research in the framework of the European project ADITEC. This last project was started in 2011 (with 42 members coming from 13 countries) with the objective of accelerating the development of new and potent immunization technologies for the new generation of human vaccines.
- Federica Sallusto and Antonio Lanzavecchia are part of the ZIKAlliance consortium (51 members from 18 different countries) that obtained a grant of €11.9 million from the Research and Innovation program Horizon 2020 of the European Union for a research project on the fight against the epidemic of the Zika virus (ZIKV) in Latin America and the Caribbean.

On May 3rd, 2016 Prof. Antonio Lanzavecchia was elected as a member of the National Academy of Sciences of the United States of America, one of the most important recognitions in the field of academic research.
Dr. Federica Sallusto, director of the Center of Medical Immunology (CIM) and head of the Cellular Immunology Laboratory of the IRB, was nominated Full Professor of Medical Immunology at ETH Zürich. This will allow for a further consolidation of the collaboration between the IRB and the Hub of Immunology of ETHZ that in turn could reinforce in a significant way its leading position in life sciences and personalized medicine on an international level.
Dr. Mariagrazia Uguccioni, head of the Chemokine and Immunity Laboratory of the IRB was nominated Professor of Histology in the Faculty of Medicine at Humanitas University in Milan.

On October 1st, 2016, Prof. Petr Cejka, professor and researcher arriving from the "Institute of Molecular Cancer Research" of the University of Zurich, started as a new Group Leader at the IRB and brought along with him both an SNF Professorship and an ERC Consolidator grant.
In addition, on May 9th, Andrea Cavalli's promotion to the position of Group Leader was approved by the Board.

In the course of 2016, the Foundation Board released a new document on the "Strategic planning of the IRB for the period 2017-2020" confirming its objectives for the next four years, consisting in the consolidation of its own structure in order to "reach a critical mass that guarantees productive stability and better possibilities of generational turnover at the level of the research groups". The mission oriented towards "conducting basic research in biomedicine to establish the third Swiss hub of biomedical research in Ticino" was also confirmed.
In that light, following the insertion of the Institute in the new Faculty of Biomedical Sciences of the Università della Svizzera italiana (USI), a new affiliation agreement was contracted with the USI that established the methods and procedures for the nomination of the Director and Group Leaders of the IRB, as well as for the hiring of professors within the new faculty.
The Director, Antonio Lanzavecchia, in the meantime was nominated Professor in the Faculty of biomedical sciences of the USI.

In addition, the different approaching phases towards the opening of the construction site for the building of the new headquarters in the "ex-military field" area were realized within the set time limits. The process for requesting the authorization for construction resulted in the issuing of a valid construction license at the beginning of this year by the Bellinzona city hall that will allow for the opening of the construction site in September of this year and for the conclusion of work by the end of 2020. Also the procedures for obtaining various public and private funding as a guarantee of the coverage of the costs of construction were completed with the concession of the promised subsidies by the City of Bellinzona and by the Canton. The Confederation also confirmed its support in covering the costs by means of subsidies based on federal law so that the funding of the work results in being insured, in line with the financing plan.
On behalf of the entire Foundation Board and all the IRB researchers, I would like to renew my gratitude to the diverse principal sponsors of the IRB, in particular to the Helmut Horten Foundation, the Ruth & Gustav Jacob Foundation, the Mäxi Foundation and the Gelu Foundation, and also to the many supporters and private donors that allow the Institute to continue to carry out its research in the best possible conditions, to train and promote young researchers, while placing in the forefront 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, June 2017

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 2015 and 2016. The main topics deal with the host defense against infectious agents and with the mechanisms of inflammatory and degenerative diseases. Below I will briefly summarize the research activities of the different groups and other notable events of the last two years.

Andrea Cavalli uses computational methods to determine the structure of folded and misfolded states of proteins, and structures of protein complexes, from minimal sets of experimental data. His group studies the mechanism of action of a novel class of compounds with STAT3 inhibitory and anti-cancer activity that work by promoting the formation of large protein aggregates as consequence of induced conformational changes. Andrea and his group are also studying the potential determinants of protein aggregation responsible for the development of light chain amyloidosis, a severe human disease that leads to severe damage of heart and kidney by deposition of aggregates formed by immunoglobulin light chains.

Petr Cejka joined the IRB in October 2016. His group uses biochemical methods to study the mechanisms of DNA repair. Cejka, has been awarded an ERC consolidator grant and an SNF Professorship, and has published his research in high-tier journals. For his contributions, he received the prestigious Friedrich Miescher Award in 2017. His current research addresses the mechanism that controls the DNA end resection, which represents the first stem in homologous recombination. Solving this mechanism has the potential to improve gene-editing technologies. Additional projects deal with the control of homologous recombination machinery, which exchanges DNA fragments between the maternal and paternal genomes during meiosis, thus contributing to genetic diversity and the link between DNA replication and repair.

Santiago González uses sophisticated *in vivo* two-photon imaging techniques to visualize cell migration and cell-cell interaction in the course of the immune response to pathogens. His laboratory characterizes the role of natural killer cells and dendritic cells in the immune response to influenza virus infection. In a study published in *Cell Reports*, his group has also described new roles for neutrophils in the immune response to vaccinia virus and for lymph node macrophages as initiators of the immune response.

Fabio Grassi studies the role of extracellular ATP as a signalling molecule capable of triggering P2 receptors on immune cells, in particular the P2X7 receptor which is upregulated in effector T cells, and has profound impact on T cell responsiveness and metabolism. In a paper published in *Cell Reports*, his group showed that T follicular helper cells by sensing microbiota-derived ATP via P2X7 promote the generation of a proficient gut ecosystem for metabolic homeostasis. They also found that P2X7 activity can limit expansion of effector T cells in ATP-rich microenvironment, thereby controlling potential T cell mediated tissue damage.

The laboratory I am leading developed high-throughput cellular screens to isolate potent and broadly neutralizing antibodies, which can be developed for prophylaxis and treatment of infectious diseases and used as tools for vaccine design. We also address fundamental aspects of the antibody response, such as the role of somatic mutations in affinity maturation and the relationship between infection and autoimmunity. In the last two years, the research in the laboratory has focused on the study of the immune response to the malaria

parasite. We have discovered that approximately 10% of malaria-exposed individuals produce antibodies of unusual structure, which are generated by the insertion into the antibody gene of a large segment of DNA encoding the LAIR1 extracellular domain that is derived from different chromosome. We also discovered that templated insertions are often found in immunoglobulin genes, pointing to a general mechanism of antibody diversification. In another study, we have identified antibodies that can confer sterilizing immunity to malaria infection and identify the target epitope as an essential component of an effective vaccine.

A new research program, spearheaded by Roger Geiger, deals with the application of proteomics, peptidomics and metabolomics to the study of human T cell differentiation and function. A study published in *Cell* has shown that L-arginine can influence metabolic networks and the lifespan of activated T cells thus enhancing their capacity to reject tumors, a finding that has important implication for tumor immunotherapy. Roger Geiger is also developing a new technology for cloning and transferring T cell receptors from tumor infiltrating T cells for adoptive T cell therapy. The availability of a cutting-edge mass spectrometry facility at the IRB is also instrumental for new peptidomics studies performed in collaboration with the laboratory of Federica Sallusto.

Maurizio Molinari studies the mechanisms that regulate quality control in the endoplasmic reticulum (ER) using novel approaches to avoid the use of toxic drugs. In particular, his group studies the role chaperones and enzymes that assist maturation of newly synthesized polypeptides and the mechanism of ER-associated degradation (ERAD). In a paper published in *Nature Cell Biology* his groups has studied endoplasmic reticulum turnover during stress recovery and showed a new role for the translocon component Sec62. These studies are relevant to understand and possibly treat rare diseases such as α1-antitrypsin deficiency and lysosomal storage diseases.

Silvia Monticelli, studies the transcriptional and posttranscriptional mechanisms that regulate gene expression in the cells of the immune system, including the role of microRNAs as well as epigenetic modifications such as the methylation of genomic DNA. In particular, in a paper published in Cell Reports her group reported how overall levels of genomic 5hmC and the activity of the TET enzymes impact on activation of mast cells, while in a recent work published in PNAS her group showed how DNA methylation-related processes are essential to restrain excessive inflammatory responses.

Federica Sallusto studies the immune response in humans using novel high throughput cell-based assays complemented with cutting-edge analytical technologies, such as next generation sequencing, single cell transcriptomics, metabolomics and proteomics. These studies address fundamental questions related to how the immune system can protect us against different classes of microbial pathogens, such as viruses, or bacteria, and to provide insights for the design of new vaccine strategies. In a study published in *Science* her group provides an extensive analysis of in vivo and in vitro CD4 T cell differentiation at the clonal level, which led to a new model of T cell differentiation. They also study cytokine gene regulation in memory subsets of healthy individuals and of patients with primary monogenic immunodeficiency. The group also studies rare autoimmune diseases such as narcolepsy, which they found to be associated to a CD4 T cell response against orexin-producing neurons.

Marcus Thelen studies the regulation of the CXCL12/CXCR4/ACKR3 axis that controls migration and positioning of hematopoietic cells, as well as tumor spread. In particular, his laboratory studies atypical

chemokine receptors (ACKRs), which mainly act as sinks and through this activity can promote modulation of cell migration. Local scavenging of chemokines not only generates chemotactic gradients, but also prevents congestion in cell trafficking. Recent work of the group has clarified the expression of these receptors and their role in signalling and cancer.

Mariagrazia Uguccioni studies chemokine modulation activities in human inflammatory diseases, tumors, and infections. Her team discovered and characterized the mechanisms leading to chemokine antagonism and synergism in the cells of the immune system. In a recent work published in the Journal of Immunology, her team has recently characterized the modification occurring in T cell during HIV 1 infection, and shown that chronic immune activation leads to an altered response to chemokines. These studies call for novel pharmacological interventions aimed at restoring chemokine receptor activities in patients with persistent infections with the aim of favoring resolution of inflammation, and a better response to vaccines.

Luca Varani combines computational structural biology with experimental validation to determine the three-dimensional structure of proteins and characterize their interactions with other molecules, with particular attention to antibody-antigen interactions in infectious diseases. His laboratory can rapidly solve structures and predict mutations that improve the efficacy of antibodies. Examples range from viral glycoproteins of Dengue and Zika viruses to prion proteins.

Laurent Perez, is leading the IRB protein production facility and contributing to research in the field of vaccines. His work has led to the production and testing of new vaccines against Human Cytomegalovirus (HCMV), bovine and human respiratory syncytial virus (BRSV and HRSV) using stabilized pre-fusion glycoproteins. He has also identified the cellular receptors for HCMV. This work provides another example of the capacity of our institute to translate basic research into new therapies.

The fruitful collaboration between researchers of the IRB with Davide Corti and coworkers at Humabs BioMed, a spin-off company of the IRB, led to new projects and publications on the role of neutralizing antibodies in severe infections such as Rabies, Influenza, Norovirus, Hepatitis B and Zika virus.

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, three IRB researchers hold a prestigious ERC Grant in recognition of the excellence and innovation of their research. The IRB researchers have received support also from the US National Institutes of Health, the Bill and Melinda Gates Foundation, and the Italian Cariplo Foundation. The research activity, which account for 43% of the IRB budget, is entirely covered by competitive grants.

The IRB continues to play an important role in teaching. Our doctorate program currently coordinated by Silvia Monticelli allowed 77 students to obtain their doctoral degree in research (PhD) at ETH and 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 Fri-

bourg through the ProDoc program. The IRB will contribute to the teaching of immunology and biology in the new Faculty of Biomedical Sciences of the Università della Svizzera italiana (USI), which is organized in collaboration with the ETH Zurich and the Univeristies of Zurich and Basel. I retired as ETH professor in February 2017 and was nominated Professor at USI. As a consequence of the affiliation with USI, the IRB faculty will be able to organize a new PhD program at USI and give the doctoral title. Federica Sallusto, leading the Center of Medical Immunology at IRB, was nominated Professor of Medical Immunology at ETHZ and will have a research laboratory at the Department of Microbiology and will teach in the Human Medicine bachelor. Mariagrazia Uguccioni was nominated Professor of Histology at the Medical faculty of Humanitas University in Milan.

Members of the IRB are often involved in the organization of scientific conferences and courses. Federica Sallusto organized, as vice chair, the Gordon Research Conference on Immunochemistry and Immunobiology in Barga, Italy. Together with Mariagrazia Uguccioni and the Dermatology Department of the University of Bern, I continue to organize at the IRB, on annual basis, the meeting of the European Society of Dermatology and Venereology (EADV). Marcus Thelen has organized the First European Chemokine and Cell Migration Conference in Villars-sur-Ollon.

In closing, with all IRB members, 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.

Antonio Lanzavecchia,
Director of the IRB

Bellinzona, June 2017

RESEARCH GROUPS

SECTION 1

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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., Proc Natl Acad Sci USA (2007), vol. 104 (23) pp. 9615-9620) and the structural characterization of the intermediate state of a protein (Neudecker et al., Science (2012), vol. 336(6079), pp. 362-36). In December 2012, he joined the IRB as an Associate Member and was appointed as Group Leader in June 2016. 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 downregulation 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 interdomain interactions and partial unfolding of STAT3 induced by STAT3i.

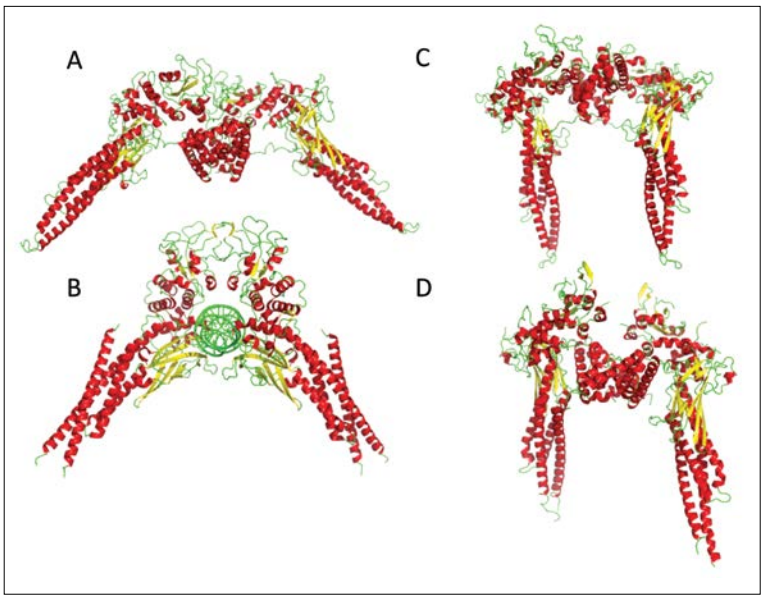

Figure 1
Computational models of the STAT3 homodimer.

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.

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

Dariusz Ekonomiuk, Maura Garofalo 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.

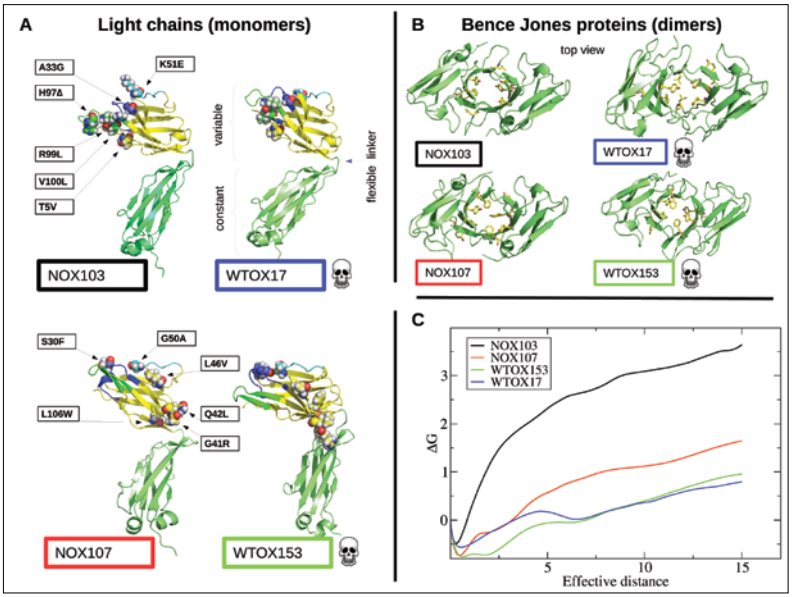

Figure 2
A) Mapping differences between toxic (WTOX17 and WTOX153) and nontoxic (NOX03 and NOX107) LC models: Constant domain is depicted in lime green; variable domain framework regions are in yellow; complementarity determining regions (CDRs): CDR1, CDR2 and CDR3 are represented in blue, cyan, and 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 interchain 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

Swiss Cancer League
Structural basis for the inhibition of STAT3 transcription factor by small molecules
2016

Swiss National Science Foundation
Identification of structural determinants of light chain amyloidosis
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Novartis Foundation for medical-biological research
Identification of potential determinants of immunoglobulin light chain amyloidosis
2015

Fondazione per la Ricerca e la Cura dei Linfomi nel Ticino
Molecular characterization of a novel class of STAT3 inhibitors
2015

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)

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Lectures and seminars

2015 Gordon Conference, Computational Aspects in Biomolecular NMR, Il Ciocco, Italy

Petr Cejka
Recombination Mechanisms

Petr Cejka

Petr Cejka received his PhD in 2004 from the University of Zurich. Petr Cejka then received a postdoctoral fellowship from the Swiss National Science Foundation to move to the laboratory of Prof. Stephen Kowalczykowski at University of California, Davis, US. Dr. Cejka received extensive training in protein biochemistry and contributed to our understanding of homologous recombination. In 2011, Petr Cejka was awarded Assistant Professorship from the Swiss National Science Foundation and returned to the University of Zurich. Dr. Cejka then established his own independent research group. The research in Dr. Cejka's laboratory is focused on various steps in the homologous recombination pathway. For his scientific achievements, Dr. Cejka received the Dr. Ernst Th. Jucker Award 2015 for contributions to cancer research. In 2016, Dr. Cejka received an ERC consolidator grant and moved to the Institute for Research in Biomedicine. In 2017, Dr. Cejka received the Friedrich Miescher Award from the LS2 section for Molecular and Cellular Biosciences.

Research Focus

Deoxyribonucleic acid (DNA) stores genetic information that contains instructions for the proper development and function of all living organisms. The integrity of DNA must be preserved during the life cycle in order to maintain cellular functions and to pass information encoded in it onto the next generation. It has been estimated that each cell in a human body acquires tens of thousands of DNA lesions per day. The sources of DNA damage may stem from the environment, such as sunlight or chemicals, or result from regular cellular processes such as metabolism. These events represent a major challenge: if left unrepaired, the lesions could block access to the genetic information and prevent faithful replication (copying) of the DNA molecule. On the other hand, incorrect repair may lead to mutations (changes in genetic information) or chromosomal aberrations (larger scale rearrangements of genetic material). These events may threaten cell viability or, in some cases, result in uncontrolled cell division (cancer).

Our research group is interested in DNA repair mechanisms from a basic research standpoint: we want to learn how these pathways operate in healthy cells and how defects lead to abnormalities and disease. Specifically, we focus on a DNA repair pathway termed homologous recombination. Homologous recombination is a highly intricate complex of processes, which repairs breaks in DNA strands. Most cells contain more than one copy of genetic information in each cell, and homologous recombination can exploit that in a very elegant manner. It can restore the integrity of the damaged DNA molecule by using genetic information stored in the identical (or homologous) copy of DNA. This process may thus restore DNA integrity in a largely accurate manner. Homologous recombination is highly conserved in evolution: the mechanism in the bacterium *Escherichia coli* or in the yeast *Saccharomyces cerevisiae* is very similar to the mechanism in human cells. This observation underlines the fundamental importance of this pathway in all kingdoms of life. Also, by using the simple organisms as research models, we can learn about homologous recombination in an experimentally more feasible setup. Our research group is using both *Saccharomyces cerevisiae* and human systems.

Team

Group Leader: Petr Cejka, PhD > petr.cejka@irb.usi.ch

Members: Roopesh Anand, PhD - Elda Cannavò Cejka, PhD - Sean Michael Howard, PhD - Lepakshi, PhD - Giordano Reginato - Nouman Khalid, PhD student.

Promotion of genetic diversity in meiosis: resolution of recombination intermediates

Roopesh Anand, Lepakshi and Petr Cejka

Promotion of genetic diversity is a key function of sexual reproduction. At the molecular level, this is controlled by the homologous recombination machinery, which exchanges (recombines) DNA fragments between the maternal and paternal genomes. During this process, joint molecules form between the 'mum' and 'dad' chromosomes, leading to intermediates termed double Holliday junctions. These joint molecules are then processed in a way that results in the physical exchange of genetic information between the two recombining chromosomes. This so-called crossover is an integral and essential part of the meiotic cell division. Results from genetic, cell biological and cytological experiments identified the Mlh1-Mlh3 heterodimer as part of a protein complex that is required for the generation of crossovers during meiotic homologous recombination. However, the mechanism of this reaction is completely unknown. The aim of our research is to analyze the behavior of the purified recombinant Mlh1--Mlh3 complex as well that of its partners in the processing of double Holliday junctions. We want to show how Mlh1-Mlh3 can cleave these structures into exclusively crossover recombination products, and therefore explain the molecular mechanism underlying the generation of diversity in meiosis.

So far, we successfully expressed and purified the yeast Mlh1-Mlh3 and human MLH1-MLH3 recombinant proteins into near homogeneity. We could show that the recombinant MutLγ is indeed a nuclease that nicks double--stranded DNA in the presence of manganese, similarly to the mismatch repair specific MutLα nuclease. MutLγ binds DNA with a high affinity, and shows a marked preference for Holliday junctions, in agreement with its anticipated activity in their processing. Specific DNA recognition has never been observed with any other eukaryotic MutL homologue. Mismatch repair specific MutLα shows no binding preference to mismatched DNA. MutLγ thus represents a new paradigm for the function of the eukaryotic MutL protein family. Unfortunately, to date, we have not seen any activity on joint molecule intermediates (such as Holliday junctions) in the presence of physiological manganese metal cofactor. This will likely require interplay of Mlh1-Mlh3 with other cellular factors (such as Exo1, Msh4-Msh5, etc.), and is the subject of vigorous research in the laboratory at present.

Recombination in DNA replication: Promoting genome stability

Lepakshi and Petr Cejka

In addition to repair double-stranded DNA breaks, homologous recombination helps to stabilize or restart replication forks in the presence of single-stranded DNA breaks or replication-blocking lesions. This likely represents the most important function of recombination, as recombination-deficient human cells can undergo only a very limited number of rounds of DNA replication. The link between stalled or collapsed replication forks and recombination is not understood. It has been inferred that the human MMS22L-TONSL complex might function in this process, but the underlying mechanism is unclear. We could show that MMS22L-TONSL binds RPA-coated single-stranded DNA, which may help recruit the complex to sites of DNA damage. By a direct interaction with the strand exchange protein RAD51, MMS22L-TONSL promotes DNA strand exchange by limiting the assembly of RAD51 on double-stranded DNA. The activity of MMS22L-TONSL then promotes replication fork reversal to protect stalled or stressed replication forks. We will further investigate how MMS22L-TONSL functions together with RAD51 to promote replication fork stability, as well as how this function interplays with other factors including SMARCAL1 and ZRANB3.
Replication fork repair by recombination must be tightly regulated so that it is only activated when needed. Unscheduled DNA recombination might lead to sister chromatid exchanges, loss of heterozygocity, genome rearrangements and other abnormalities, and must be thus tightly controlled. The ultimate goal of our experiments is to understand how MMS22L-TONSL regulates recombination specifically upon replication fork stalling. Our research is anticipated to shed light on the link between DNA replication and repair.

First steps in homologous recombination: DNA end resection

Roopesh Anand, Elda Cannavò Cejka, Sean Michael Howard and Petr Cejka

Homologous recombination is initiated by the nucleolytic degradation (resection) of the 5'-terminated DNA strand of the DNA break. This leads to the formation of 3'-tailed DNA, which becomes a substrate for the strand exchange protein RAD51 and primes DNA synthesis during the downstream events in the recombination pathway. DNA end resection thus represents a key process that commits the repair of DNA breaks into recombination. Research from multiple laboratories established that DNA end resection is in most cases a two-step process. It is initiated by the nucleolytic degradation of DNA that is at first limited to the vicinity of the broken DNA end. This is carried out by the Mre11-Rad50-Xrs2 (MRX) complex and Sae2 proteins in yeast, and MRE11-RAD50-NBS1 (MRN) and CtIP proteins in human cells (Figure 1). We could reconstitute these reactions *in vitro*, and demonstrated that Sae2 and CtIP stimulate a cryptic endonuclease activity within the yeast MRX or human MRN complex, respectively. The activity of Sae2/CtIP is absolutely dependent on its phosphorylation. The reconstituted DNA clipping reaction allows us to investigate the mechanism of this process as well as its regulation by posttranslational modifications and additional protein co-factors. Specifically, we investigate how phosphorylation stimulates Sae2 and CtIP on the mechanistic level. We also study how the endonuclease and exonuclease activities of the MRE11-RAD50-XRS2/NBS1 complex are regulated by the individual subunits and other proteins, including the BRCA1-BARD1 complex.

Downstream of MRX-Sae2 and MRN-CtIP, which process only a limited length of DNA, DNA end resection is further catalyzed by Sgs1-Dna2 or Exo1 in yeast and BLM-DNA2, WRN-DNA2 or EXO1 in human cells. We are interested how these nucleases take over the nuclease function from the MRE11-RAD50-NBS1/

XRS2 complex. Also, we study how these protein complexes form molecular machines that are uniquely capable to resect long lengths of DNA, which is required for homologous recombination. We are specifically interested in the Dna2 enzyme, and could show that both yeast Dna2 and human DNA2 possess a cryptic helicase activity. We now investigate how the motor activity of Dna2 promotes DNA end resection, as well as how it is regulated in cells. Finally, as some of these enzymes are upregulated in various human cancers, we are also searching for small molecules capable to inhibit these pathways as a potential cancer therapy approach.

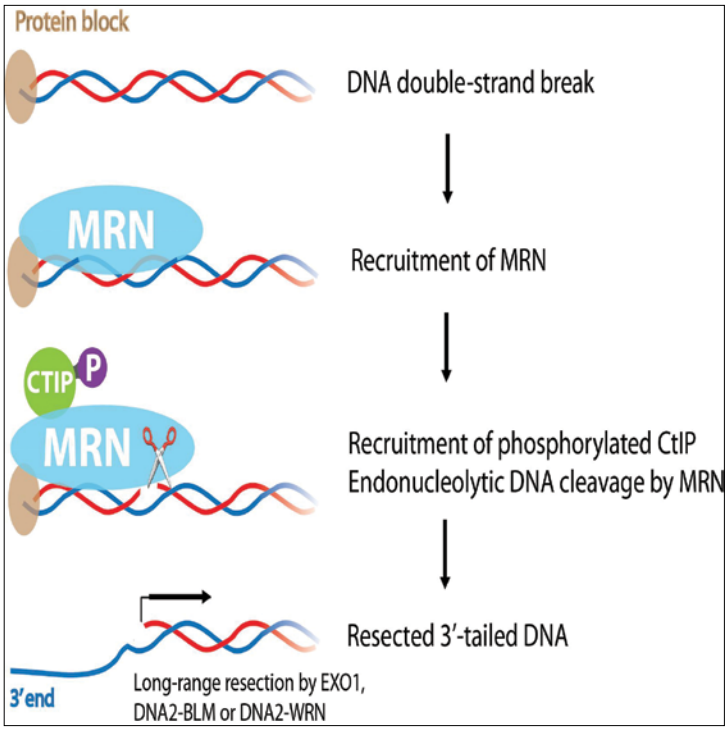

Figure 1
Processing of protein blocked DNA ends by the MRE11-RAD50-NBS1 complex. Phosphorylated CtIP protein activates a cryptic endonuclease activity of MRE11, which cleaves 5'-terminated DNA in the vicinity of a protein blocked DNA break. This initiates DNA double-strand break repair by homologous recombination. From Anand et al, Mol Cell, 64, 2016.

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Publications

The Mre11-Nbs1 Interface Is Essential for Viability and Tumor Suppression.
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Phosphorylated CtIP Functions as a Co-factor of the MRE11-RAD50-NBS1 Endonuclease in DNA End Resection.
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Lectures and Seminars

Seminar at Weatherall Institute of Molecular Medicine, University of Oxford
"Initiation of DNA double-strand break repair in yeast and humans"
Oxford (UK) / December 2016

Seminar at IFOM
"Initiation of DNA double-strand break repair in yeast and humans"
Milan (IT) / November 2016

Seminar at ETHZ
"Initiation of DNA double-strand break repair in yeast and humans"
Zurich (CH) / May 2016

Abcam conference on Mechanisms of Genetic Recombination
"Mechanisms of DNA end resection in yeast and humans"
Alicante (ES) / May 2016

Seminar at Biozentrum
"Mechanisms of Homologous recombination"
Basel (CH) / April 2016

Conference
"Sae2 promotes the nuclease activity of MRX to initiate recombination. Structure-Specific Endonucleases in Genome Stability"
Brno (CZ) / November 2015

Seminar at Ludwig Maximilan University of Munich
"Mechanisms of homologous recombination"
Munich (DE) / November 2015

Seminar at Nordea Center for Healthy Aging, University of Copenhagen
"Mechanisms of homologous recombination"
Copenhagen (DK) / September 2015

Faseb Conference on Genetic Recombination and Genome Rearrangements
"Human MMS22L-TONSL Complex Promotes Homologous Recombination"
Steamboat Springs, CO (US) / July 2015

Keynote lecture at the Cancer Network Zurich Retreat
"Mechanisms of homologous recombination"
Emmetten (CH) / April 2015

Gordon Research Conference, Mammalian DNA repair
"Human MMS22L-TONSL Complex Promotes Homologous Recombination"
Ventura, CA (US) / February 2015

Santiago F. González

Infection and Immunity

Santiago F. González

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

Research Focus

The primary focus of my lab is to study the initiation of the inflammatory response using *in vivo* imaging techniques. The main areas of my research interest include the innate and adaptive immune responses to respiratory pathogens, and the mechanisms by which such viruses and bacteria fight the host immune system. The initial response of the body to infection involves a series of events characterized by the rapid up-regulation and recruitment of effectors molecules and cells, which facilitate the elimination of the pathogen and the restoration of homeostasis. In our group we characterize the initial mechanisms of the host response directed to contain the infection and to initiate a protective response. The combination of the *in vivo* and in vitro perspectives will contribute to the better understanding of the immune response to disease challenges, allowing the design of more effective ways to enhance the host immune response.
We are currently using state-of-the-art imaging techniques such as 2-photon intravital microscopy, and confocal microscopy to address some of the aforementioned questions. In addition, we also use some classic imaging techniques, such as electron microscopy, in order obtained structural information of the tissue or cell of interest.

Team

Group Leader: Santiago F. González, PhD, PhD > santiago.gonzalez@irb.usi.ch
Members: Yagmur Farsakoglu, PhD student - Miguel Palomino, PhD student - Diego Pizzagalli, PhD student - Tommaso Virgilio, 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 in order to function optimally NK cells require priming by other immune cells, such as macrophages and dendritic cells. 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 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. NK activation reaches a peak at 12 h post vaccination, which is characterised by a prominent IFNγ secretion. At that time, NK cells show stable and prolonged interactions with activated CD169+ macrophages both in the medullary and subcapsular sinus regions of LN (Figure 1). Furthermore, we could observe a clear correlation between NK cell recruitment and the presence of retained vaccine. However, NK function change with time. Later time point analyses indicate a differential activity of NK cells focusing on their killing capacity. On day 5 post vaccination NK cells peak the expression of degranulation markers such as CD107a and perforin. Additionally, elimination of NK cells resulted in decreased antibody responses to influenza vaccine, demonstrating the implication of NK cells in the adaptive response. Overall, our findings designate a time-dependent functional heterogeneity in NK cell responses to influenza vaccination. Along this line, this study focuses on characterising further the mechanisms by which the time-dependent differential activity of NK cells shape the innate versus adaptive immune response to influenza vaccine.

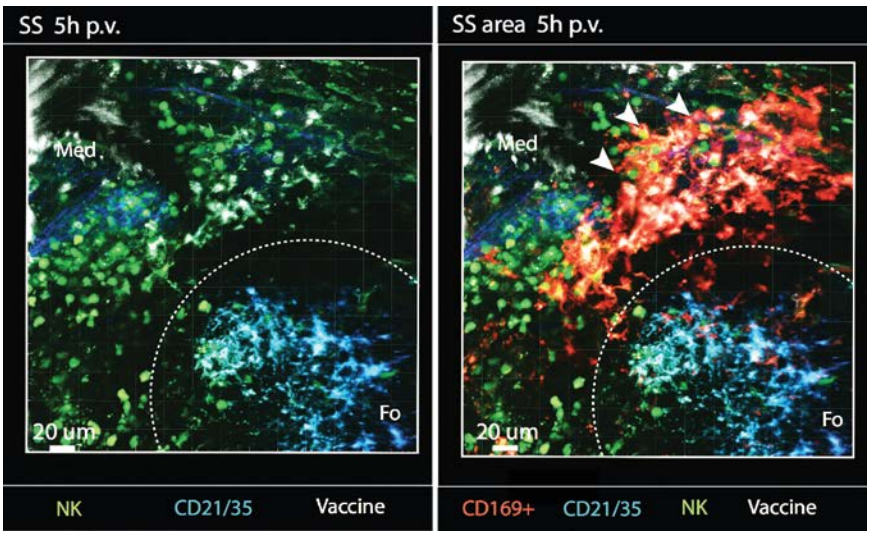

Figure 1.
2-Photon intravital micrograph showing NK cells (green) interacting with CD169+ macrophages (red) in the popliteal lymph node.

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

Diego Pizzagalli and Santiago F. González

Recent advances in intravital imaging have allowed the study of the behaviour of cells with remarkable depth and resolution in organs and tissues, making microscopy videos rich sources of biomedical knowledge. To this extent, specific computational tools are needed to automatically generate a quantitative and meaningful description of the observed phenomenon from the acquired imaging data. However, state of the art software and methods for the analysis of microscopy videos exhibit limitations when studding immune cells due to the high plasticity, frequent contacts and variable movement patterns during their interaction. Indeed, measures and statistics commonly used to describe the behaviour of motile cells, such as the total displacement length or the directionality, are highly affected by errors in cell surface reconstruction and tracking, requiring manual correction.

In this work we have proposed an imaging-based Systems Biology approach, representing the microscopy scene as a graph where cells are nodes and their transactions are edges (Figure 2). Such a data structure supports the usage of computational techniques such as global optimization and machine learning to describe interactions and to predict and track the movement of immune cells in a meaningful and robust way. In order to automatically create such a graph from microscopy images, a specific algorithm working at single-pixel (voxel for 3d data) has been developed. Such an algorithm further allows investigating motility at sub-cellular level. However, in order to have a sound basis for evaluating the performances of tracking algorithms on videos of immune cells in broad experimental conditions and sites of imaging, an extended ground-truth dataset is required. Therefore, we have set up an online database of two-photon microscopy videos where immune cells have been manually tracked (Leukocyte Tracking Database - www.ltdb.info).

This work is done in collaboration with Prof. Dr. Marcus Thelen (IRB) and Prof. Dr. Rolf Krause, Institute of Computational Science, Università della Svizzera italiana (CH).

The Leukocyte Tracking Database has been created with the contribution of Dr. Mempel, Massachusetts General Hospital (MGH), Boston (US) and Dr. Stein, Theodor Kocher Institute (TKI), University of Bern (CH).

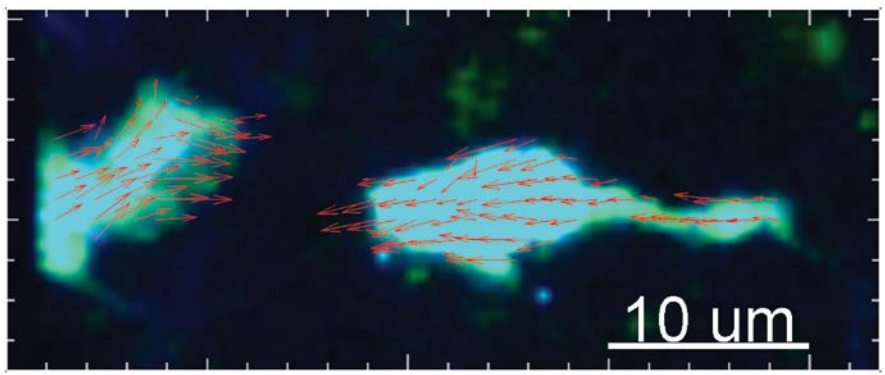

Figure 2
Movement estimation on two fluorescently-labeled neutrophils observed by two-photon intravital microscopy. Red arrows indicate the pixel displacement.

Role of Inflammatory Dendritic Cells in the upper respiratory tract during influenza infection

Miguel Palomino, Tommaso Virgilio and Santiago F. González

Influenza virus is responsible of high morbidity and mortality worldwide and a leading cause of death amongst young children, old people and the immuno-compromised. Despite the fact that the initial phase of the infection occurs in the upper respiratory tract, little is known about the immune reaction that follows infection and how it affects the outcome of the disease. Our research focused on elucidating the role of the inflammatory response in the mucosa of the trachea during early stages following influenza infection. Using 2-photon microscopy we observed a the dense network of dendritic cells (DC) located under the mucosa of the trachea from mice, which increased significantly their number at day 3 post infection (p.i.) with influenza virus (Figure 3). The characterization of the tracheal subgroups of DC according to the expression of different surface markers indicated that at day 3 p.i. the majority of the infiltrated DC correspond to the inflammatory phenotype characterised by the expression of CD45+ / MHCII+ / CD11c+ / CD11b+ / Ly6c. Our efforts focus on investigating the relevance of the inflammatory dendritic cells (IDC), a monocyte-derived subtype, in the early response against influenza virus. Our data suggested that IDC together with the resident interstitial macrophages are involved in the initiation of the inflammatory response soon after viral infection. We observed that the activation and recruitment of the invariant γδ T cells in the tracheal mucosa was also dependent on the presence of this type of DC. Furthermore, we observed that IDC recognize the pathogen via the c-type lectin receptor SIGN-R1 that has the capacity to bind influenza virus and is highly express on these cell type. Moreover, the absence of this receptor impaired the initial chemokine production leading to a significant lower presence and activation of γδ T cells. Finally, we observed that the blockade of SIGN-R1 increased the susceptibility to influenza infection and it correlated with lower levels of anti-influenza antibodies. All this data suggested, that IDCs are key initiators of the immune reaction to influenza infection through the detection of viral particles and the secretion of chemokines that contribute to the recruitment and activation of other innate cells in the tracheal mucosa.

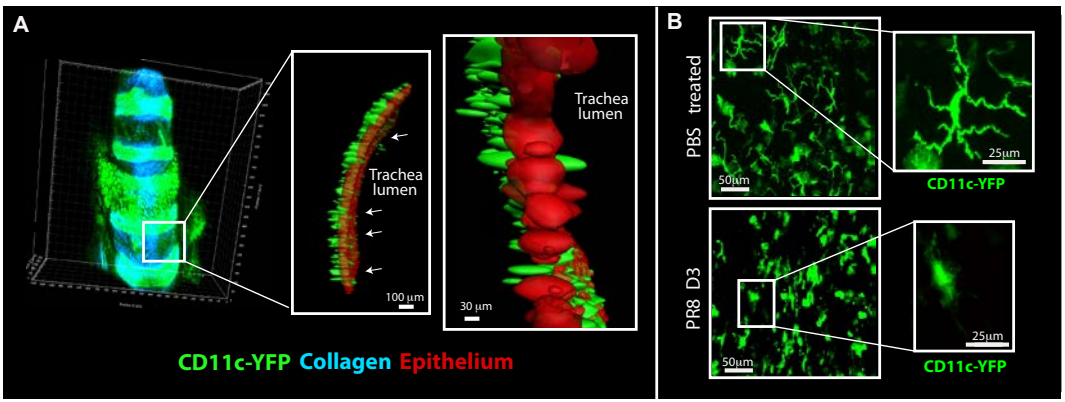

Figure 3. *A) Intravital 2-photon micrograph reconstruction showing the projection from a CD11c+ DC into the tracheal luminal space. B) Intravital 2-photon micrographs showing the changes in the morphology of the CD11c+ DC before (upper part) and after (loers part) infection with influenza virus.*

Role of neutrophils in the adaptive immune response to vaccinia virus

Miguel Palomino Segura, Mauro Di Pilato and Santiago F. González

Despite their abundance and physiological importance, not much is known about the role of the neutrophils in promoting adaptive immune responses. Recently, different studies have stressed 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. However, a recent publication by di Pilato and colleagues showed that during the infection with an attenuated vaccinia virus enhanced neutrophil trafficking to the infection site correlated with an increased CD8 T-cell adaptive immune response. The infection with this attenuated virus, which lacks three specific viral genes, increased the expression of several cytokines/chemokines that promoted a higher migration of two neutrophil populations (Nα and Nβ) to the infection site. Further analysis of identified an activated Nβ cell subtype with APC function that migrated to the spleen. However, the way that this neutrophil subset might be promoting specific adaptive immune responses has not been yet addressed. Our working hypothesis is that antigen transport to the spleen results from the uptake of apoptotic cells by Nβ cells in the site of infection followed by rapidly transport of processed antigens to this organ. There, this Nβ cells are able to interact with CD8 T cells directly, which then respond/proliferate specifically. The aim of this project is to characterise the migration patterns of the infiltrated neutrophils *in vivo* in the spleen and its interaction with CD8T cells using 2-photon intravital microscopy. This technique will allow us to specifically monitor cell to cell interactions and to address the novel role as APCs of the Nβ cells during the vaccinia infection.

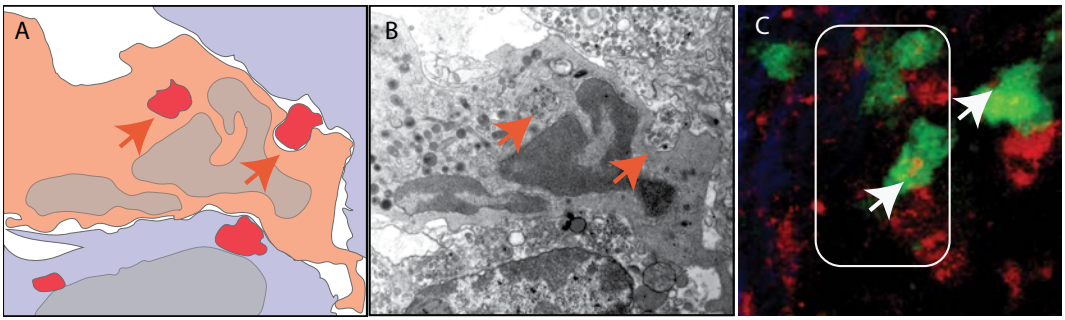

Figure 4. *A) Schematic representation of an electron micrograph (B) and 2-photon snapshot (C) showing the capture and internalication of virus (arrows) by newly recruited neutrophils.*

Lymph node macrophages as initiators of the immune response

Yagmur Farsakoglu 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 and TLR7 dependent. 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. 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-1α and IFN-β 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 LN, 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.

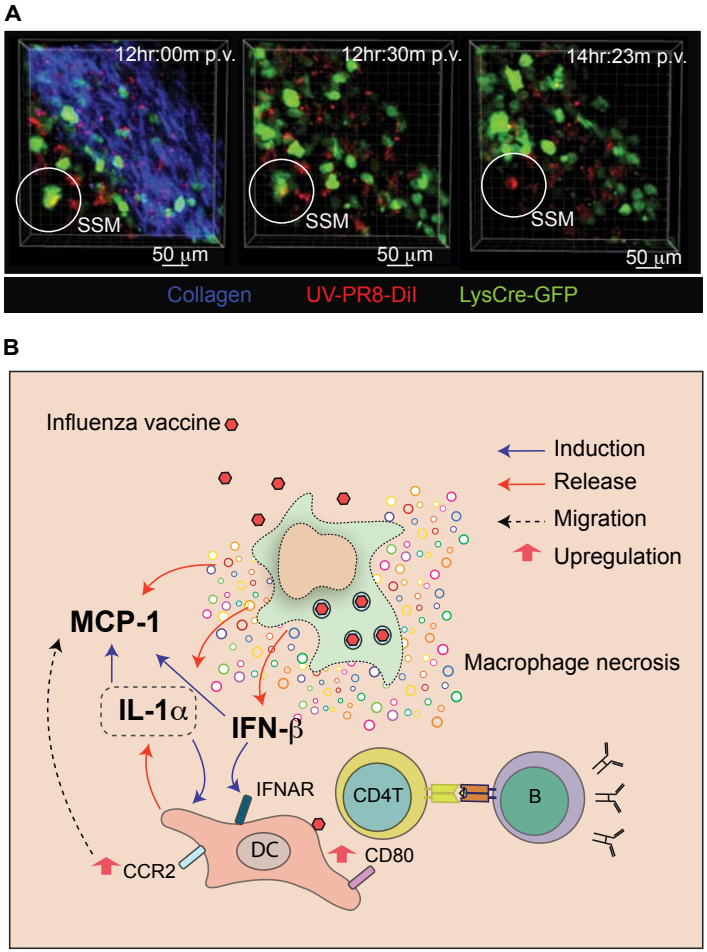

Figure 5.
A) Intravital 2PM sequence from LysMCre-GFP reporter mice (green) shows subcapsular sinus macrophages (SSM) disappearance at 12 h p.v. with DiI-labelled UV-PR8 (red)

B) Proposed model of the role of SSM in the activation of dendritic cells (DC) and the initiation of the inflammatory response in the mouse popliteal LN following vaccination with UV-PR8. SSM capture influenza vaccine, become activated and secrete IFN-β that induces the production of IL-1α by DC. IL-1α induces the up regulation of CD80, a T cell costimulatory molecule. Additionally, DC are attracted to the subcapsular sinus area in a process that is mediated by the chemokine receptor CCR2, expressed on DC and the chemoattractant MCP-1 that is released by SSM and other DC after stimulation with IL-1α and/or IFN-β.

Lymphatic migration of metastatic cancer cells to the lymph node

Tommaso Virgilio and Santiago F. González

The initial phase of a tumour is frequently associated with cancer cell dissemination through the lymphatic system. Upon entry in the lymphatic vessels, tumour cells moved towards the draining lymph node dLN (Figure 6A). Invasion of the cancer cells to the LN is one of the first signs of metastatic spread. However the dynamics of the spreading of tumour cells in this organ and the initial interaction with the immune compartment have not been fully address. The study of this interaction is critical to understand the cellular mechanisms that lead to the successful growth of the tumour.
The goal of this project is to evaluate *in vivo* using intravital 2-photon microscopy the arrival to the lymph node of different type of fluorescently labelled tumour cells via the afferent lymphatics (Fig 6B). Once the tumour cell reach the lymph node, we will study the migratory behaviour in the different areas and the interaction with the stromal compartment and the major phagocytic populations (macrophages and dendritic cells) (Fig 6C, E). Understanding the dynamics of this interaction will contribute to the efficacious design of new immunotherapies against melanoma and breast cancer.

This work is done in collaboration with Professors Jonas Fuxe and Mikael Karlsson from the Karolinska Institute (SE).

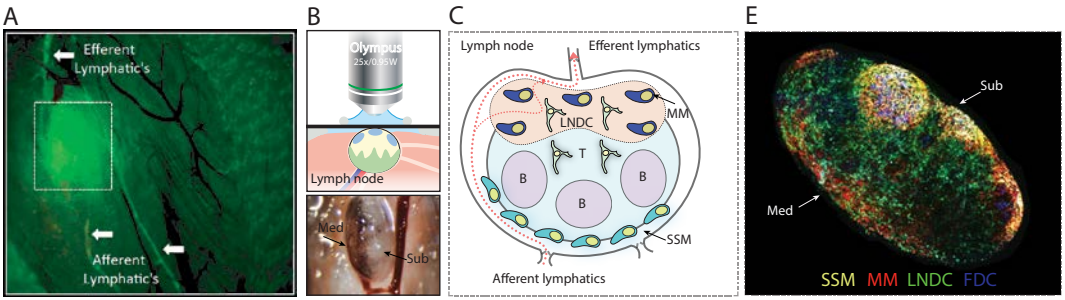

Figure 6
A) Micrograph of a popliteal region of a Prox1-GFP transgenic mice showing the relative position of the popliteal lymph node (LN) with respect to the blood and lymphatic vessels (afferent and efferent).
B) Intravital 2-photon microscopy set-up showing the microsurgery in the popliteal area. Once exposed, the popliteal LN is protected and movies are generated using the 2-photon microscope (upper pannel). The lower pannel shows a macroscopic view of the medullary (Med) and the subcapsular sinus regions (Sub) of a popliteal lymph node after microsurgery.
C) Schematic drawing of a popliteal LN showing the position of the major phagocytic populations, SSM (Subcapsular Sinus Macrophages), MM (Medullary Macrophages) and LNDC (Lymph Node resident Dendritic Cells).
E) 2-photon micrograph showing a 3D reconstruction of a popliteal LN with the mayor phagocytic populations FDC indicates the follicular regions.

Funding

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148183 / 2014-2016

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

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 / 2013-2017

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Visiting Scientist
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Appl Microbiol Biotechnol. 2016; 100:10585-10595.

Lectures and seminars

Toll 2015. Targeting Innate Immunity
Lecture: "Lymph node macrophages as first-line mediators of the immune response"
Marbella (ES) /01.09.15

Invited lecture at the master in BioSciences from Homeostasis to Disease (ENS Lyon)
Lecture: "Trafficking of antigen in the lymphatic system following vaccination"
Lyon (FR) /26.05.16

13th International Conference on Innate Immunity
Lecture: "Macrophages as initiators of the immune response in the lymph node"
Rhodes (GR) /27.06.16

International Congress of Immunology
Lecture: "Characterization of the inflammatory response in the upper respiratory track following influenza infection"
Melbourne (AU) /24.08.16

International Congress of Immunology
Lecture: "Macrophage death following influenza vaccination promotes IL-1α-dependent dendritic cell activation and antigen presentation in draining lymph nodes."
Melbourne (AU) /26.08.16

EADV meeting Bellinzona 2016
Lecture: "Macrophage-associated secretion of IL-1αpromotes dendritic cell function and induces B cell response after influenza vaccination"
Bellinzona (CH) /01.12.16

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 full professor of Biology at the University of Milan. The research in the lab is focused on the purinergic control of T cell response. At the moment, particular efforts are dedicated to defining the role of extracellular ATP and P2X7 receptor in regulating mucosal adaptive immune response as well as mutualism with intestinal commensals.

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. Moreover, extracellular ATP is massively released upon tissue injury and acts as damage associated molecular pattern (DAMP) via P2 receptors stimulation. 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). The P2X7 receptor subtype is robustly upregulated in effector T cells, and has profound impact on T cell responsiveness and metabolism. Prolonged P2X7 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. We are currently investigating the role of P2X7 in limiting T cell-mediated immunopathological damage as well as mucosal immunity.

Team

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

ATP released by gut commensals in the regulation of mucosal immunity, microbiota composition and host metabolism

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

The ATP-gated ionotropic P2X7 receptor regulates T follicular helper (Tfh) cell abundance in the Peyer's patches (PPs) of the small intestine; deletion of *P2rx7*, encoding for P2X7, in Tfh cells results in enhanced IgA secretion and binding to commensal bacteria. We have shown that Tfh cells activity is important for generating a diverse bacterial community in the gut and that sensing of microbiota-derived extracellular ATP via P2X7 promotes the generation of a proficient gut ecosystem for metabolic homeostasis. The results of this study indicate that Tfh cells play a role in host-microbiota mutualism beyond protecting the intestinal mucosa by induction of affinity-matured IgA and suggest that extracellular ATP constitutes an inter-kingdom signaling molecule important for selecting a beneficial microbial community for the host via P2X7-mediated regulation of B cell help (Figure 1).

*** *Perruzza L. et al.***
Cell Reports. 2017, 18: 2566-2575.

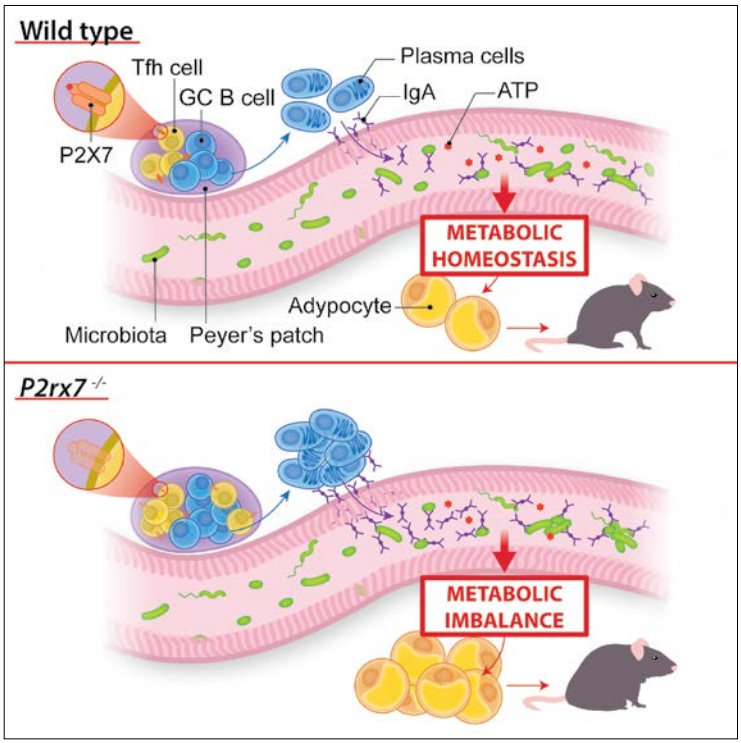

Figure 1
Control of adaptive immune response by ATP released by gut commensals promotes a beneficial gut ecosystem.
Stimulation of the P2X7 receptor by ATP released by bacteria controls Tfh cells abundance and secretory IgA response against commensals in the small intestine. In P2rx7$^{-/-}$ mice, the increased abundance of Tfh cells results in enhanced IgA secretion, alterations of gut microbiota and metabolic imbalance..

Modulation of effector/memory T cell responsiveness by P2X7 activity

Andrea Romagnani, Elsa Rottoli, Michela Perotti 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. *P2rx7*$^{-/-}$ TEM cells are characterized by a bioenergetic advantage with respect to wild-type (WT) cells. Morphometric analysis of mitochondria revealed altered cristae and increased mitochondrial mass in *P2rx7*$^{-/-}$ TEM cells. Consistent with these morphometric changes, Western Blot analysis showed an impairment of the autophagic flux in *P2rx7*$^{-/-}$ TEM cells by a decrease in both lipidated form of LC3 protein, namely LC3-II and LC3-I and accumulation of p62 protein. Microarray gene expression analysis showed that *P2rx7*$^{-/-}$ TEM cells clustered together and separately from WT cells. Among differentially expressed genes we identified cyclin-dependent kinase inhibitor 1A (*Cdkn1a*), encoding for p21$^{Waf1/Cip1}$, as a transcript upregulated in WT cells. P21 regulates progression through G_1 to S phase in mammalian cells. To address whether P2X7 signaling directly regulated *Cdkn1a* expression we stimulated WT TEM cells with BzATP as a selective P2X7 agonist. This resulted in significant increase in *Cdkn1a* transcripts with respect to unstimulated cells and this increase was abrogated by the selective P2X7 antagonist A-438079. These results suggest that P2X7 activity limits expansion of TEM cells in ATP-rich microenvironment (e.g. during inflammation), thereby controlling potential T cell mediated tissue damage.

The P2X7 receptor in the control of T follicular helper cells in autoimmunity

Caterina E. Faliti, Lisa Perruzza, Elsa Rottoli and Fabio Grassi

Excessive signaling or altered control of T follicular helper (Tfh) cells can lead to generation of autoantibodies and autoimmune manifestations. In mice, intra-peritoneal injection of pristane induces a lupus-like syndrome characterized by polyclonal hypergammaglobulinemia, autoantibodies and glomerulonephritis. We have shown that lack of the ATP gated ionotropic P2X7 receptor in Tfh cells enhanced B cell helper activity and generation of autoantibodies, promoted IFN-γ secretion and worsened the outcome of this syndrome. Conversely, lack of P2X7 did not alter the response of Tfh cells to ovalbumin as an exogenous antigen. Circulating Tfh cells from patients with systemic lupus erythematosus (SLE) but not primary anti-phospholipid syndrome (PAPS) were hyporesponsive to P2X7 stimulation and resistant to P2X7-mediated inhibition of cytokine-driven proliferation. Moreover, CD4 naïve cells from SLE were not sensitive to P2X7-mediated control of differentiation to Tfh. These data suggest that P2X7 constitutes a selective regulator of Tfh cells function to limit autoimmunity in SLE pathogenesis.

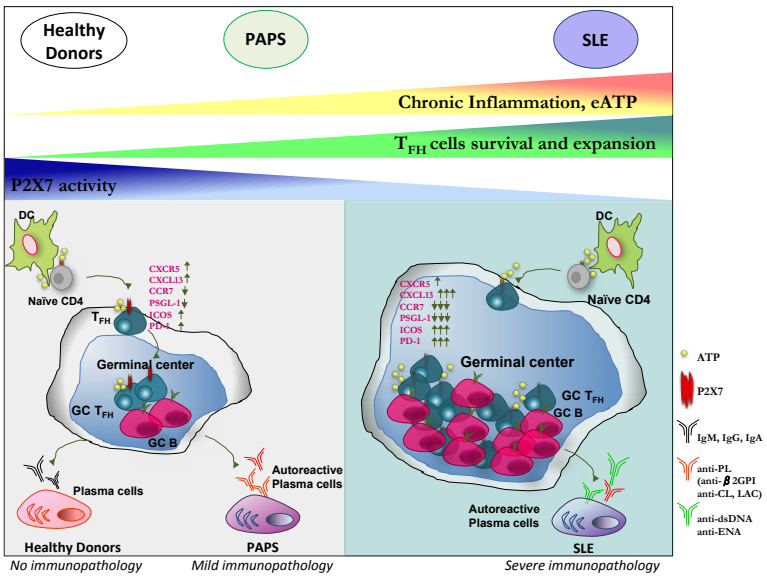

Figure 2
P2X7 activity regulates Tfh cells expansion to limit autoimmunity in systemic lupus erythrmatosus.
Tfh cells from patients with systemic lupus erythematosus (SLE) but not primary antiphospholipid syndrome (PAPS) are hyporesponsive to P2X7 stimulation. This results in enhanced Tfh cells function, generation of anti-double-stranded (ds) DNA, anti-extractable nuclear antigens (ENA) antibodies and severe immunopathology. Conversely, in primary anti-phospholipid syndrome (PAPS), P2X7 activity limits Tfh cells expansion; autoantibodies reactivity is limited to phospholipid (PL), β2-glycoprotein I (β2GPI), cardiolipin (CL) and lupus anticoagulant (LAC).

Purinergic antagonism in muscular dystrophy

Lisa Perruzza and Fabio Grassi

***Gazzerro E. et al.** **
The American Journal of Pathology. 2015; *185: 3349-3360.*

Infiltration of immune cells and chronic inflammation substantially affect skeletal and cardiac muscle degeneration in Duchenne muscular dystrophy. The P2X7 receptor has a prominent role in regulating immune system physiology and contributes to inflammasome activation also in muscle cells. We have shown that *in vivo* blockade of the extracellular ATP/P2X purinergic signaling pathway by periodate-oxidized ATP (oATP) delayed the progression of the dystrophic phenotype and dampened the local inflammatory response in *mdx* mice, a spontaneous mouse model of dystrophin deficiency. Reduced infiltration of leukocytes and macrophages and decreased expression of IL-6 were revealed in the muscles of oATP-treated *mdx* mice. Concomitantly, an increase in Foxp3^{+} immunosuppressive regulatory T cells was observed and correlated with enhanced myofiber regeneration. Moreover, we detected reduced concentrations of profibrotic cytokines, including transforming growth factor-β and connective tissue growth factor, in muscles of oATP-treated *mdx* mice. The improvement of inflammatory features was associated with increased strength and reduced necrosis, thus suggesting that pharmacologic purinergic antagonism altering the adaptive immune component in the muscle infiltrates might represent a promising therapeutic approach in Duchenne muscular dystrophy.

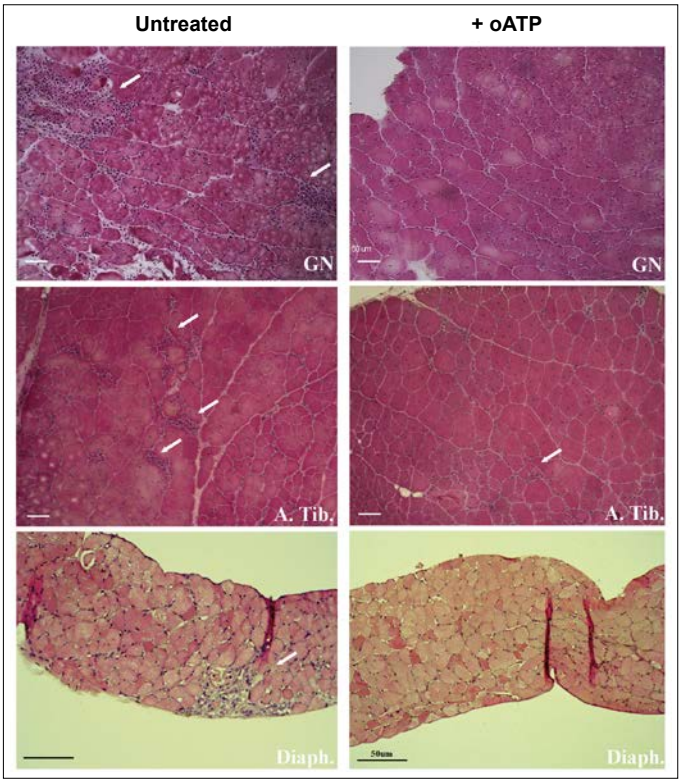

Figure 3
Periodate-oxidized ATP (oATP) improves reactive inflammatory infiltrates in mdx-4cv mice.
Frozen gastrocnemii (GN), anterior tibialis (A.Tib.), and diaphragm (Diaph.) muscle tissue sections from *mdx*-4cv mice either untreated or treated with oATP were collected at the fourth week of treatment and stained with standard H&E technique. White arrows indicate inflammatory infiltrates.

Role of CXCR4-mediated bone marrow colonization in central nervous system infiltration by T-cell acute lymphoblastic leukemia

Tanja Rezzonico-Jost, Andrea Romagnani, Lisa Perruzza and Fabio Grassi

Infiltration of the central nervous system is a severe trait of T cell acute lymphoblastic leukemia (T-ALL). Inhibition of CXC chemokine receptor 4 (CXCR4) significantly ameliorates T-ALL in murine models of the disease; however, signaling by CXCR4 is important in limiting the divagation of peripheral blood mononuclear cells out of the perivascular space into the central nervous system parenchyma. Therefore, inhibition of CXCR4 potentially may untangle T-ALL cells from retention outside the brain. We have shown that leukemic lymphoblasts massively infiltrate cranial bone marrow, with diffusion to the meninges without invasion of the brain parenchyma, in mice that underwent xenotransplantation with human T-ALL cells or that developed leukemia from transformed hematopoietic progenitors. We tested the hypothesis that T-ALL neuropathology results from meningeal infiltration through CXCR4-mediated bone marrow colonization. Inhibition of leukemia engraftment in the bone marrow by pharmacologic CXCR4 antagonism significantly ameliorated neuropathologic aspects of the disease. Genetic deletion of CXCR4 in murine hematopoietic progenitors abrogated leukemogenesis induced by constitutively active Notch1, whereas lack of CCR6 and CCR7, which have been shown to be involved in T cell and leukemia extravasation into the central nervous system, respectively, did not influence T-ALL development. We hypothesize that lymphoblastic meningeal infiltration as a result of bone marrow colonization is responsible for the degenerative alterations of the neuroparenchyma as well as the alteration of cerebrospinal fluid drainage in T-ALL xenografts. Therefore, CXCR4 may constitute a pharmacologic target for T cell acute lymphoblastic leukemia neuropathology.

*** *Rezzonico Jost T. et al.***
Journal of Leukocyte Biology.
2016; 99: 1077-1087.

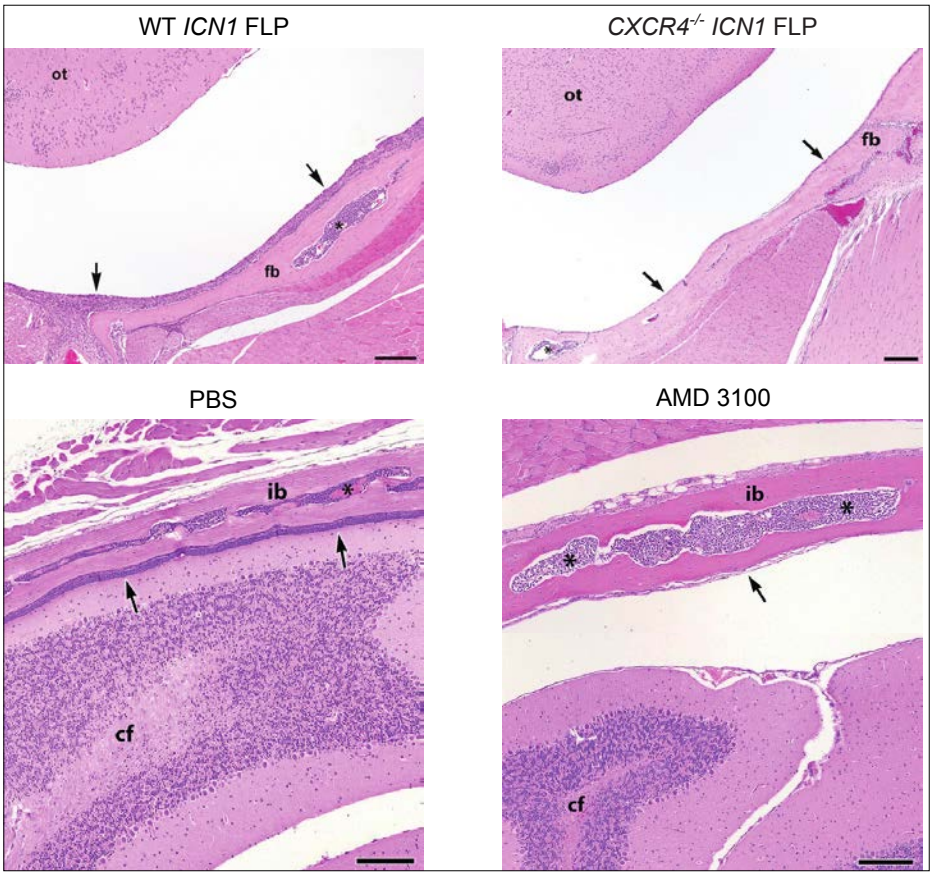

Figure 4

Deletion or pharmacological antagonism of CXCR4 inhibits bone marrow engraftment and meningeal infiltration by T cell acute lymphoblastic leukemia.

Upper panels. Head of mice transplanted with *ICN1*-transduced fetal liver progenitors (FLP). Left panel shows leukemic infiltrates replacing the hematopoietic BM in the cranial diploe (*) with diffuse invasion, and expansion of underlying meninges, including dura and arachnoid mater (arrows), in mice that underwent transplantation with *ICN1*-transduced wild-type FLPs. Right panel shows normal hematopoietic BM without leukemic component in cranial diploe (*) in mice that underwent transplantation with *ICN1*-transduced *CXCR4*$^{-/-}$ FLP. The meningeal layers (arrows) are free from any leukemic infiltrate. fb, frontoparietal bone; ot, olfactory tubercle. Scale bars = 120 µm. *Lower panels*. Head from NSG mice xenotransplanted with T-ALL cells from human patient. Left panel shows leukemic infiltrates replacing the hematopoietic BM in the cranial diploe (*) with invasion into the underlying meninges (arrows) in mice treated with PBS. Right panel shows that leukemic infiltrates in AMD-3100–treated mice are confined to the hematopoietic BM in the cranial diploe (*) without invasion into the underlying meninges (arrow). The expansion of the arachnoid space with separation of skull and neuroparenchyma represents a common artefact associated with the preparation of paraffin sections from the whole head. cf, cerebellar folia; ib, intraparietal bone. Scale bars = 160 µm

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

Andrea Romagnani, Tanja Rezzonico-Jost, Elsa Rottoli, Michela Perotti, Michele Proietti and Fabio Grassi

The *melastatin-like transient-receptor-potential-7* protein (TRPM7), harboring a cation channel and a serine/threonine kinase, has been implicated in thymopoiesis and cytokine expression. By means of a TRPM7 kinase-dead mutant (*TRPM7*$^{R/R}$) we have shown that the enzymatic activity of the receptor is not essential for thymopoiesis. However, gut intraepithelial T cells (IELs) are absent in *TRPM7*$^{R/R}$ mice due to an intrinsic defect in CD103 transcriptional regulation. The defect in gut colonization by T cells results in significantly reduced MHCII expression in intestinal epithelial cells. TRPM7 kinase activity controls TGF-β-induced CD103 expression as well as Th17 cell differentiation via regulation of SMAD2, but is dispensable for regulatory T (Treg) cell differentiation. Importantly, we found that the TRPM7 kinase activity promotes gut colonization by alloreactive T cells in acute graft-versus-host-disease. Thus, our results unravel a fundamental role of TRPM7 kinase in T cell function and suggest a therapeutic potential of kinase inhibitors in averting acute graft-versus-host-disease.

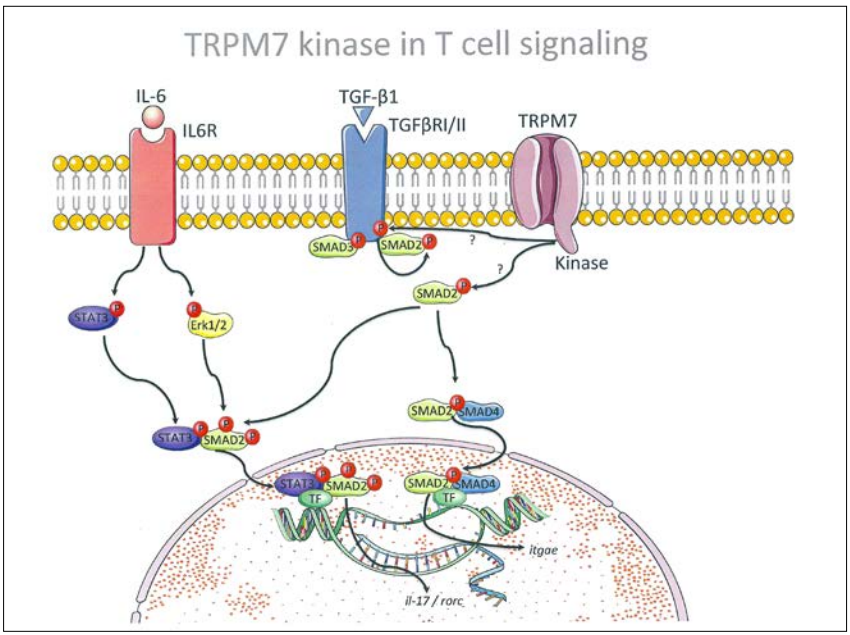

Figure 5
Model of TRPM7 kinase function in T cell signaling.
Upon binding of TGF-β1, the TGFβ receptor complex initiates the phosphorylation of the C terminal SXS motif of SMAD2 and SMAD3. We propose an additional mechanism by which the TRPM7 kinase phosphorylates SMAD2 directly, once it is anchored to the plasma membrane. Phosphorylated SMAD2 interacts with SMAD4 and promotes the transcription of *Itgae*, *Il-17* and *Rorc* genes. IL-6-dependent STAT3 as well as Erk1/2-dependent SMAD2 linker phosphorylation pathways are unaltered in TRPM7 kinase deficient T cells.

Mucosal immunity in the pathogenesis of Omenn Syndrome

Fabio Grassi

Omenn syndrome (OS) is caused by hypomorphic Rag mutations and characterized by a profound immunodeficiency associated with autoimmune-like manifestations. Both in humans and mice, OS is mediated by oligoclonal activated T and B cells. The role of microbial signals in disease pathogenesis is debated. We have shown that hypomorphic *Rag2*^{R229Q} knock-in mice developed an inflammatory bowel disease affecting both the small bowel and colon. Lymphocytes were sufficient for disease induction, as intestinal CD4 T cells with a Th1/Th17 phenotype reproduced the pathological picture when transplanted into immunocompromised hosts. Moreover, oral tolerance was impaired in *Rag2*^{R229Q} mice, and transfer of wild-type (WT) regulatory T cells ameliorated bowel inflammation. Mucosal immunoglobulin A (IgA) deficiency in the gut resulted in enhanced absorption of microbial products and altered composition of commensal communities. The *Rag2*^{R229Q} microbiota further contributed to the immunopathology because its transplant into WT recipients promoted Th1/Th17 immune response. Consistently, long-term dosing of broad-spectrum antibiotics (ABXs) in *Rag2*^{R229Q} mice ameliorated intestinal and systemic autoimmunity by diminishing the frequency of mucosal and circulating gut-tropic CCR9^{+} Th1 and Th17 T cells. Remarkably, serum hyper-IgE, a hallmark of the disease, was also normalized by ABX treatment. These results indicate that intestinal microbes may play a critical role in the distinctive immune dysregulation of OS.

***Rigoni R. et al.** **
The Journal of Experimental Medicine. 2016; 213: 355-375.

Funding

Swiss National Science Foundation
Purinergic control of adaptive immunity by P2X7 receptor
310030_159491 / 2015-2018

Fondazione Gelu
CXCR4 as a therapeutic target in T acute lymphoblastic leukemia
2015-2017

COST
Role of ATP gated ionotropic P2X7 receptor in regulating adaptive mucosal immunity, host-microbiota mutualism and sytemic metabolism
2017-2018

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Publications

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Am J Pathol. 2015; 185:3349-3360.

Lectures and Seminars

Department of Pharmacology, University of Milan
Milan, Italy / 06.02.2015

17th International Congress of Mucosal Immunology
Berlin, Germany / 14-18.07.2015

1st COST meeting "Ion channels and immune response"
Warsaw, Poland / 24-25.09.2015

Rusconi Lecture 2015, Fondazione Rusconi
Varese, Italy / 18.11.2015

Keystone Symposium "Purinergic signaling"
Vancouver, Canada / 24-28.01.2016

Keystone Symposium "T follicular helper cells and germinal centers"
Monterey, USA / 26.02-01.03.2016

3rd COST meeting "Ion channels and immune response"
Zagreb, Croatia / 15-16.09.2016

GSK Vaccines
Siena, Italy / 23.09.2016

Antonio Lanzavecchia

Immune Regulation

Antonio Lanzavecchia

Lanzavecchia is an immunologist known for his work on antigen presentation, T cell activation, immunological memory and human monoclonal antibodies. Born in Italy, Lanzavecchia obtained a medical degree from the University of Pavia, where he specialized in paediatrics and in infectious diseases. He worked at the Basel Institute for Immunology and, since 2000, is the founding director of the Institute for Research in Biomedicine in Bellinzona, Switzerland. From 2009 to 2016 has been professor of human immunology at the Federal Institute of Technology and since 2017 is Professor at the Faculty of Biomedical Sciences of the Università della Svizzera italiana (USI). Lanzavecchia received the EMBO Gold Medal and the Cloetta Prize and is a member of the EMBO, of the Swiss Academy of Medical Sciences and of the US National Academy of Sciences.

Research Focus

Lanzavecchia's laboratory investigates the mechanisms of antibody-mediated resistance to infectious diseases. They use high-throughput cellular screens to isolate potent and broadly neutralizing antibodies, which can be developed for prophylaxis and treatment of infectious diseases and used as tools for vaccine design. They also address fundamental aspects of the antibody response, such as the role of somatic mutations in affinity maturation and the relationship between infection and autoimmunity. Recently, they discovered in malaria-immune individuals a new mechanism of antibody diversification that involves insertions of templated DNA sequences into immunoglobulin genes. A new research program, spearheaded by Roger Geiger, deals with the application of proteomics and metabolomics to the study of human T cell differentiation and function.

Team

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A LAIR1 insertion generates broadly reactive antibodies against malaria variant antigens

Joshua Tan, Kathrin Pieper*, Luca Piccoli*, Mathilde Foglierini, Roger Geiger, David Jarrossay, Chiara Silacci Fregni, Blanca Fernandez-Rodriguez, Sonia Barbieri, Peter Bull, Antonio Lanzavecchia *equal contribution*

Variant surface antigens expressed on the surface of P. falciparum-infected erythrocytes are important targets of naturally acquired immunity against malaria, but their high number and variability provide the pathogen with a powerful means of escape from host antibodies. Although broadly reactive antibodies against these antigens could be useful as therapeutics and in vaccine design, their identification has proven elusive. We isolated from two immune donors human monoclonal antibodies that recognize erythrocytes infected by different *P. falciparum* isolates and opsonize these cells by binding to members of the RIFIN family. These antibodies acquired broad reactivity through a novel mechanism of insertion of a large DNA fragment between the V and DJ segments (Figure 1). The insert, which is both necessary and sufficient for binding to RIFINs, encodes the entire 98 amino acid collagen-binding domain of LAIR1, an immunoglobulin superfamily inhibitory receptor encoded on chromosome 19. In each of the two donors studied, the antibodies are produced by a single expanded B-cell clone and carry distinct somatic mutations in the LAIR1 domain that abolish binding to collagen and increase binding to infected erythrocytes. These findings illustrate, with a biologically relevant example, a novel mechanism of antibody diversification by templated DNA insertion in the Ig genes and demonstrate the existence of conserved epitopes that may be suitable candidates for the development of a malaria vaccine.

*** Tan. et al.**
Nature. 2016, 7:105-9.

Collaborators: Davide Corti and Siro Bianchi, Humabs BioMed, Bellinzona (CH); Peter Bull, Kevin Marsh and co-workers, KEMRI-Wellcome Trust Research Programme, Kilifi (KE).

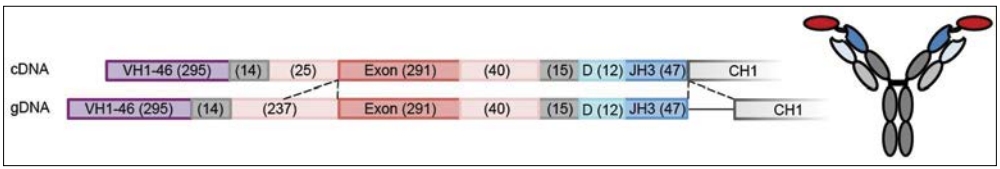


Figure 1
A templated *LAIR1* insertion between V and DJ generates antibodies carrying the LAIR1 domain on the tip of CDR3.

Public antibodies to malaria antigens generated by two LAIR1 insertion modalities

Kathrin Pieper, Joshua Tan*, Luca Piccoli*, Mathilde Foglierini, Sonia Barbieri, Yiwei Chen, Chiara Silacci-Fregni, Tobias Wolf, David Jarrossay, Marica Anderle, Federica Sallusto and Antonio Lanzavecchia *equal contribution*

We previously described two donors in whom a LAIR1 insertion between the V and the DJ segments generated, through somatic mutations, broadly reactive antibodies against RIFINs, a type of variant antigen expressed on the surface of Plasmodium falciparum-infected erythrocytes (IEs). To investigate how frequently such antibodies are produced in response to malaria infection, we screened plasma from two large cohorts of individuals living in malaria-end emic regions. We report that 5-10% of malaria-exposed individuals, but none of the European blood donors tested, have high levels of LAIR1-containing antibodies that dominate the response to infected erythrocytes. By analyzing the antibody-producing B cell clones at the protein, cDNA and gDNA level, we characterized additional LAIR1 insertions between the V and DJ segments and discovered a second insertion modality whereby the LAIR1 exon encoding the extracellular domain and flanking intronic sequences are inserted into the switch region (Figure 2). By exon shuffling, this mechanism leads to the production of bispecific antibodies in which the LAIR1 domain is precisely positioned at the elbow between the VH and CH1 domains. Additionally, in one donor the gDNA encoding the VH and CH1 domains was deleted, leading to the production of a camel-like LAIR1-containing antibody. Sequencing of the switch regions of memory B cells from European blood donors revealed frequent templated insertions originating from transcribed genes that, in rare cases, comprised exons with orientation and frame compatible with expression. Collectively, these results reveal different modalities of LAIR1 insertion that lead to public and dominant antibodies against IEs and suggest that insertion of templated DNA represents an additional mechanism of antibody diversification that can be selected in the immune response against pathogens and exploited for B cell engineering.

Collaborators: Claudia Daubenberger, STI, Basel (CH); Peter Crompton, NIAID, Rockville, MA (US); Peter Bull, KEMRI-Wellcome Trust, Kilifi (KE).

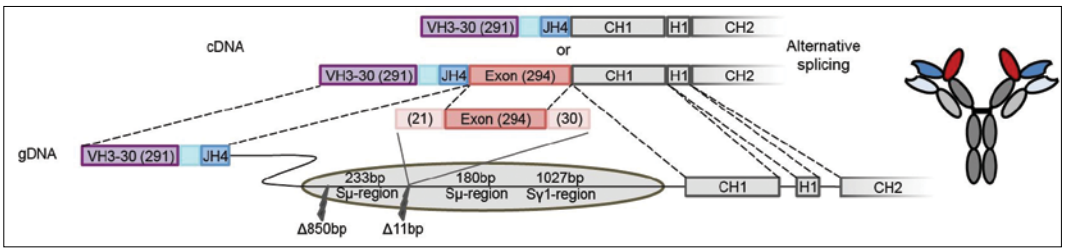
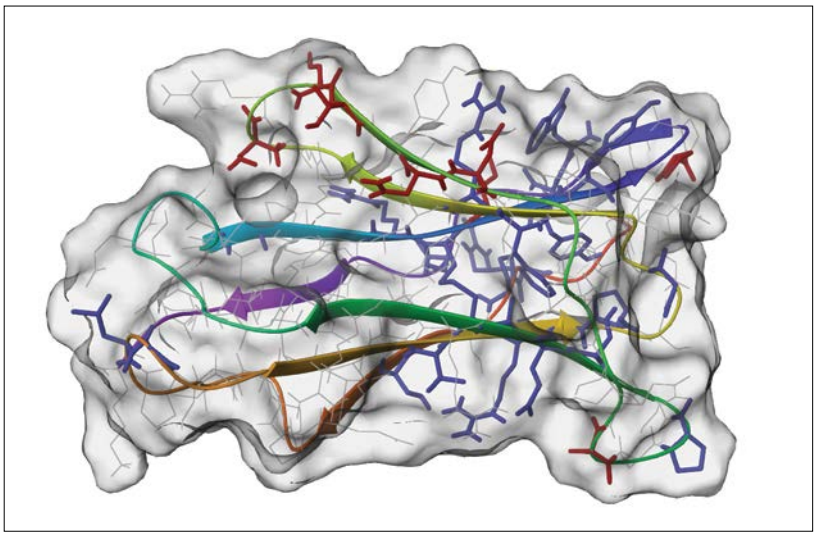


Figure 2
A templated *LAIR1* insertion in the switch region generates bispecific antibodies with the LAIR1 in VH-CH1 elbow region.

Figure 3
Somatic mutations in the *LAIR1* domain abolish collagen binding and increase binding to infecter erythrocytes.



A Public lineage of antibodies that potently block malaria infection

Joshua Tan, Luca Piccoli, Chiara Silacci, Sonia Barbieri, and Antonio Lanzavecchia

The RTS,S vaccine comprises the repeat and C-terminal region of the *P. falciparum* circumsporozoite protein (CSP) and shows significant, albeit modest, efficacy in conferring sterilizing immunity to malaria. To investigate the basis of antibody-mediated protection from malaria infection, we studied the antibody response of African volunteers that were immunized with irradiated sporozoites and were protected from challenge with live sporozoites. Memory B cells from these individuals were immortalized and B cell supernatants were screened by flow cytometry for their capacity to stain intact sporozoites in order to identify all the antibodies that may be able to bind the infectious parasite. All sporozoite-specific antibodies isolated from 5 protected donors recognized CSP, demonstrating the immunodominance of this protein. The antibodies were mapped to different regions of CSP, namely the NANP repeat, the N-terminal and C-terminal regions, and were tested for their sporozoite-blocking activity both in vitro and *in vivo* using PfGFP-luc parasites and the FRG huHEP liver-chimeric mouse model. Remarkably, the most effective antibodies as determined in the *in vivo* protection assay bound to an N-terminal 18 amino acid peptide that is not present in the RTS,S vaccine. Furthermore, in 4 different donors, these antibodies use the same VH gene, VH3-30. Our findings delineate a public lineage of antibodies that bind to the CSP N-terminal region and potently block malaria infection and suggest that the 18-mer peptide should be considered as an important component in a sterilizing malaria vaccine.

Collaborators: Claudia Daubenberger and co-workers, Swiss Tropical Institute, Basel (CH); Brandon Sack, CIDR, Seattle (US).

Platelet-derived growth factor-α receptor is a cellular receptor for human cytomegalovirus

Anna Kabanova, Jessica Marcandalli, Daniele Lilleri, Chiara Silacci-Fregni, Mathilde Foglierini, Blanca Fernandez-Rodriguez, Roger Geiger, Antonio Lanzavecchia and Laurent Perez

***Kabanova et al.** **
Nat. Microbiol. 2016, 1:16082.

Human cytomegalovirus encodes at least 25 membrane glycoproteins that are found in the viral envelope. While gB represents the fusion protein, two glycoprotein complexes control the tropism of the virus: the gHgLgO trimer is involved in the infection of fibroblasts, and the gHgLpUL128L pentamer is required for infection of endothelial, epithelial and myeloid cells. Two reports suggested that gB binds to ErbB1 and PDGFRα. However, these results do not explain the tropism of the virus and were recently challenged. Here, we provide a 19 Å reconstruction for the gHgLgO trimer and show that it binds with high affinity through the gO subunit to PDGFRα, which is expressed on fibroblasts but not on epithelial cells. We also provide evidence that the trimer is essential for viral entry in both fibroblasts and epithelial cells. Furthermore, we identify the pentamer, which is essential for infection of epithelial cells, as a trigger for the ErbB pathway. These findings help explain the broad tropism of human cytomegalovirus and indicate that PDGFRα and the viral gO subunit could be targeted by novel anti-viral therapies.
Collaborators: Davide Corti, Humabs BioMed, Bellinzona (CH); Giuseppe Gerna, IRCCS Policlinico San Matteo, Pavia (IT); Massimiliano Pagani, INGM Milano (IT); Peter Kwong and coworkers, VRC, NIAID, Bethesda (US).

Antibody-guided vaccine design

Antonio Lanzavecchia, Alexander Frühwirth, Debora Pinna, Laurent Perez

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

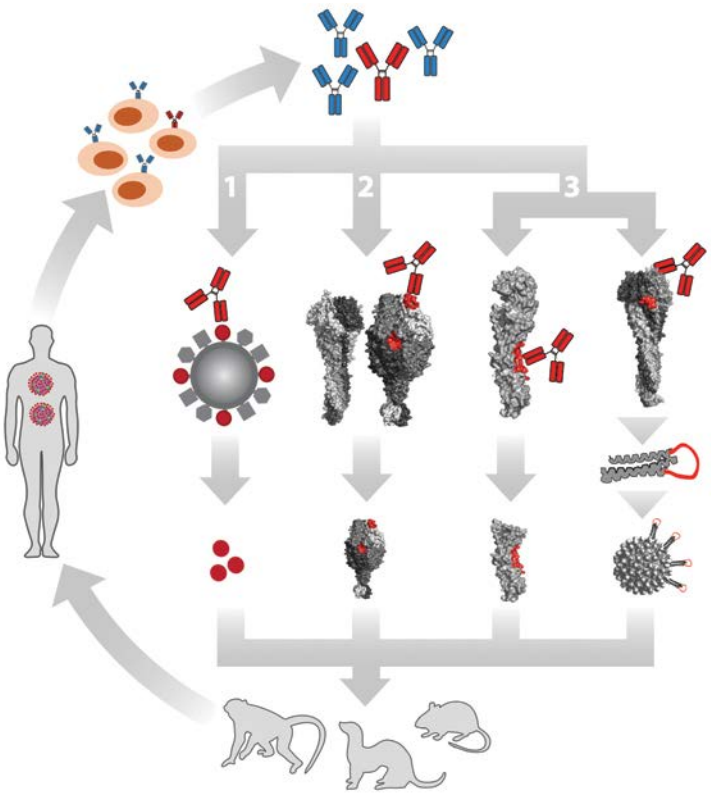
***Lanzavecchia et al.** **
Curr. Opin. Immunol. 2016, 41:62-7.

***Zhang et al.** **
npj Vaccines. 2017, 2:7.

In the last decade, progress in the isolation of human monoclonal antibodies provided an innovative approach to the identification of protective antigens and the design of vaccines capable of eliciting effective B-cell immunity. Using a target-agnostic approach we screen human memory B cell repertoires to identify antibodies of highest neutralizing potency and breadth and we use such antibodies to identify the target antigens which are then produced in a recombinant form and tested in preclinical animal models. This approach, that we have defined as "analytic vaccinology" is particularly useful in the case of complex pathogens such as herpes viruses, parasites and bacteria. A relevant example is the identification of the HCMV pentamer as the target of the most potent neutralizing antibodies and its production as an effective vaccine. More recent work form our laboratory involves the identification of RIFINs as target of broadly reactive antibodies against blood stage malaria parasites. A second approach that can be defined as "structural vaccinology" involves the use of neutralizing antibodies to define the protective epitopes within a given protein. A relevant example from our laboratory are the identification of conserved epitopes in pre-fusion RSV and MPV glycoproteins as well as the finding that 90% of neutralizing antibodies bind to the pre-fusion, but not to the post-fusion conformation (Corti et al, Nature 2013). The pre-fusion-specific antibodies are used as probes to test stabilized pre-fusion glycoproteins produced in the laboratory of Peter Kwong at the Vaccine Research Center in Bethesda. A first fruit of this collaboration is the production of a bovine RSV F vaccine that efficiently protects calves from BRSV infection (Zhang et al npj Vaccines 2017). We are pursuing a similar strategy for parainfluenza viruses (PIV1-4). In collaboration with David Baker at the Institute for protein design in Seattle we are testing the immunogenicity of nanoparticles carrying stabilized pre-fusion F proteins.

Collaborators: Davide Corti, Humabs BioMed, Bellinzona (CH); Peter Kwong, VRC, NIAID, Bethesda (US) and with David Baker, Institute for protein design, University of Washington, Seattle (US).

Figure 4
Antibody-guided vaccine design. 1) analytic vaccinology; 2 Structure-based vaccinology and 3) Epitope-focused vaccinology.



Structure and function analysis of an antibody recognizing all influenza A subtypes

Alexander Fruehwirth, Mathilde Foglierini, Chiara Silacci, Blanca Fernandez-Rodriguez, Debora Pinna and Antonio Lanzavecchia.

***Kallewaard et al.** **
Cell. 2016, 166:596-608.

This work was done in collaboration with Davide Corti and coworkers, Humabs BioMed (Bellinzona); JoAnn Suzich, Qing Zhu and coworkers, MedImmune LLC, Gaithersburg (USA) and John Skehel, Steve Gamblin and coworkers, The Francis Crick Institute, London (UK). Influenza virus remains a threat because of its ability to evade vaccine-induced immune responses due to antigenic drift. Here, we describe the isolation, evolution, and structure of a broad-spectrum human monoclonal antibody, MEDI8852, effectively reacting with all influenza A hemagglutinin (HA) subtypes. MEDI8852 uses the heavy-chain VH6-1 gene and has higher potency and breadth when compared to other anti-stem antibodies. MEDI8852 is effective in mice and ferrets with a therapeutic window superior to that of oseltamivir. Crystallographic analysis of Fab alone or in complex with H5 or H7 HA proteins reveals that MEDI8852 binds through a coordinated movement of CDRs to a highly-conserved epitope encompassing a hydrophobic groove in the fusion domain and a large portion of the fusion peptide, distinguishing it from other structurally characterized cross-reactive antibodies. The unprecedented breadth and potency of neutralization by MEDI8852 support its development as immunotherapy for influenza virus-infected humans. In this study, we also report the genealogical analysis of B cell clones giving rise to pan-influenza neutralizing antibodies (FI6 and FY1). The results delineate a group 1 prime, group 2 boost strategy for the induction of pan-influenza neutralizing antibodies.

Public lineages, rapid affinity maturation and epitope-dependent effector functions characterize the human antibody response to Ca09 Hemagglutinin

Alexander Fruehwirth, Philipp Paparoditis, Kathrin Pieper, Esther Katelaars, Mathilde Foglierini, Chiara Silacci, Blanca Fernandez-Rodriguez Sonia Barbieri and Antonio Lanzavecchia.

The antibody response against influenza hemagglutinin (HA) comprises anti-head antibodies, which are primarily subtype-specific and target multiple antigenic sites, and anti-stem antibodies that cross-react with different subtypes. By analyzing the global antibody response at the polyclonal and monoclonal level using virus escape mutants, we dissected the response to multiple sites, including a novel, conserved site in the membrane-proximal region. The capacity of the monoclonal antibodies to mediate different effector functions was dependent on their site specificity. Isolation and mapping of more than 200 monoclonal anti-head antibodies revealed multi-donor lineages that use VH2-70/VL3 and bind to the Sa/Sb site. These antibodies are potent neutralizers and do not need affinity maturation in contrast to other anti-head antibodies, which matured rapidly through just a few mutations. These findings identify a public lineage of high affinity germline antibodies that develop in different individuals in response to last pandemic H1N1 virus.

High-avidity IgA protects the intestine by enchaining growing bacteria

Luca Piccoli, Blanca Fernandez-Rodriguez, Sonia Barbieri, Costanza Casiraghi, and Antonio Lanzavecchia

This work was done in collaboration with Wolf Hard, Emma Slack and coworkers at the Institute of Microbiology ETH Zurich (CH) and with Davide Corti and coworkers at Humabs BioMed, Bellinzona (CH). Vaccine-induced high-avidity IgA can protect against bacterial enteropathogens by directly neutralizing virulence factors or by poorly defined mechanisms that physically impede bacterial interactions with the gut tissues ('immune exclusion'). IgA-mediated cross-linking clumps bacteria in the gut lumen and is critical for protection against *S. Typhimurium*. However, classical agglutination, which was thought to drive this process, is efficient only at high pathogen densities (≥108 non-motile bacteria per gram). In typical infections, much lower densities (10^0-10^7 colony-forming units per gram) of rapidly dividing bacteria are present in the gut lumen. Here we show that a different physical process drives formation of clumps *in vivo*: IgA-mediated cross-linking enchains daughter cells, preventing their separation after division, and clumping is therefore dependent on growth. Enchained growth is effective at all realistic pathogen densities, and accelerates pathogen clearance from the gut lumen. Furthermore, IgA enchains plasmid-donor and -recipient clones into separate clumps, impeding conjugative plasmid transfer *in vivo*. Enchained growth is therefore a mechanism by which IgA can disarm and clear potentially invasive species from the intestinal lumen without requiring high pathogen densities, inflammation or bacterial killing. These results also reveal an untapped potential for oral vaccines in combating the spread of antimicrobial resistance.

* ***Moor K. et al.***
Nature. 2017, 544:498-502.

Protective monotherapy against lethal Ebola virus infection by a potently neutralizing antibody

Chiara Silacci, Blanca Fernandez-Rodriguez, Laurent Perez, Federica Sallusto, and Antonio Lanzavecchia

Ebola virus disease in humans is highly lethal, with case fatality rates ranging from 25 to 90%. There is no licensed treatment or vaccine against the virus, underscoring the need for efficacious countermeasures. We ascertained that a human survivor of the 1995 Kikwit Ebola virus disease outbreak maintained circulating antibodies against the Ebola virus surface glycoprotein for more than a decade after infection. From this survivor, we isolated monoclonal antibodies (mAbs) that neutralize recent and previous outbreak variants of Ebola virus and mediate antibody-dependent cell-mediated cytotoxicity in vitro. Strikingly, monotherapy with mAb114 protected macaques when given as late as 5 days after challenge. Treatment with a single human mAb suggests that a simplified therapeutic strategy for human Ebola infection may be possible.

This work was done in collaboration with Nancy Sullivan and co-workers at the Vaccine Research Center, NIAID, Behtesda, MA (US) and Davide Corti and co-workers at Humabs BioMed Bellinzona (CH).

* ***Corti et al.***
Science. 2016, 351:1339-42.

* ***Misasi et al.***
Science. 2016, 351:1343-6.

Prophylactic and postexposure efficacy of a human monoclonal antibody against MERS-CoV

Blanca Fernandez-Rodriguez, Mathilde Foglierini, and Antonio Lanzavecchia.

Corti et al. *
PNAS. 2015, 112:10473-8.

Middle East Respiratory Syndrome (MERS) is a highly lethal pulmonary infection caused by a previously unidentified coronavirus (CoV), likely transmitted to humans by infected camels. There is no licensed vaccine or antiviral for MERS, therefore new prophylactic and therapeutic strategies to combat human infections are needed. In this study, we describe, for the first time, to our knowledge, the isolation of a potent MERS-CoV-neutralizing antibody from memory B cells of an infected individual. The antibody, named LCA60, binds to a novel site on the spike protein and potently neutralizes infection of multiple MERS-CoV isolates by *interfering* with the binding to the cellular receptor CD26. Importantly, using mice transduced with adenovirus expressing human CD26 and infected with MERS-CoV, we show that LCA60 can effectively protect in both prophylactic and postexposure settings. This antibody can be used for prophylaxis, for postexposure prophylaxis of individuals at risk, or for the treatment of human cases of MERS-CoV infection. The fact that it took only 4 mo from the initial screening of B cells derived from a convalescent patient for the development of a stable CHO cell line producing neutralizing antibodies at more than 5 g/L provides an example of a rapid pathway toward the generation of effective antiviral therapies against emerging viruses.

This work was done in collaboration with Luca Varani and Federica Sallusto, IRB, Davide Corti, Humabs BioMed Bellinzona, Ralph Baric, UNC Chapel Hill (US) and Maria Zambon, PHE, London (UK).

Generation of secondary idiotypes through Immunoglobulin Light Chain pairing in the context of Light Chain Amyloidosis

Sara Ravasio, Dariusz Ekonomiuk, Sonia Barbieri, Maura Garofalo, Andrea Cavalli, and Antonio Lanzavecchia

Antibody diversity is generated by V-(D)-J recombination, somatic hypermutation and heavy (HCs) and light chains (LCs) pairing. To avoid production of autoantibodies, self-reactivity is tested at the level membrane-bound B cell receptors i.e. at the level of VH/VL pairs. In contrast, there are no mechanisms that may control the potential self-reactivity of L chain dimers, which are produced in excess by plasma cells. To address if free LCs could be endowed with antigen-binding properties, we investigated Light Chain Amyloidosis (AL), a disease characterized by clonal production of aggregation-prone LCs, that can provoke the deposition of amyloid fibrils and damage target organs, mainly heart and kidney. In particular, we hypothesize that the self-reactivity of L chain dimers may be a factor that promotes tissue deposition and determines organ tropism. We also consider the possibility that somatic mutations could influence LCs toxicity and aggregation propensity although our in vitro and in silico data suggest that most amyloidogenic LCs have biochemical characteristics, stability and aggregation propensity which do not differ from their germline counterparts. Interestingly, mutations in proximity of the linker region were found to strongly impact on the properties of LCs such as heavy chains pairing. To address a potential tissue specificity of L chain dimers, we measure binding of recombinant human LCs to cell lysates. Our findings indicate that free LCs have binding properties different from that of the original antibody, that may favor tissue deposition whenever serum concentration arises to high levels as it occurs in AL. Preliminary data suggest LCs ability to bind to mouse and human tissues revealing patterns compatible with amyloid deposition in patients. Further efforts are needed to clarify the interactome of free LCs and to elucidate the molecular determinants of LC-driven toxicity.

A restricted T-cell response sustains the generation of neutralizing anti-Natalizumab antibodies in patients with multiple sclerosis

Luca Piccoli, and Antonio Lanzavecchia

Natalizumab is a humanized monoclonal antibody approved for the treatment of relapsing-remitting multiple sclerosis (MS). It binds to the α4-integrin, preventing leukocyte migration into the central nervous system and reducing inflammation. Unfortunately, about 10% of the patients develop neutralizing anti-Natalizumab antibodies that reduce the clinical efficacy of the treatment. By immortalizing IgG memory B cells from an MS patient treated with Natalizumab, we isolated 30 anti-drug monoclonal antibodies that bind to Natalizumab with very high affinity. The antibodies use different V genes, suggesting that Natalizumab induces a polyclonal antibody response. Such response is anti-idiotypic, as the antibodies lose binding to different Natalizumab variants where three or more CDRs were substituted with the CDRs of the original human antibody scaffold. Interestingly, 60% (18/30) of the antibodies were able to inhibit binding of Natalizumab to α4-integrin expressed on T cells in vitro, an activity that was acquired through somatic mutations and correlated with a very slow antibody dissociation rate. Natalizumab-reactive T cells were also isolated from the same patient by stimulation in vitro with overlapping peptides covering the entire sequence of the variable region of the Natalizumab heavy and light chain. Epitope mapping and T-cell receptor gene sequencing revealed the presence of three distinct T cell clones, all recognizing the CDR2 region of the Natalizumab light chain. In conclusion, our results suggest that Natalizumab treatment in MS induces the generation of a restricted T-cell response which is sufficient to sustain the generation of a wide number of neutralizing anti-Natalizumab antibodies.

Social network architecture of human immune cells unveiled by quantitative proteomics

Roger Geiger, Tobias Wolf, David Jarrossay, Federica Sallusto, and Antonio Lanzavecchia

This work was done in collaboration with Matthias Mann, Felix Meissner and co-workers at the Max Planck Institute of Biochemistry, Bayern (D). The immune system is unique in its dynamic interplay between numerous cell types. However, a system-wide view of how immune cells communicate to protect against disease has not yet been established. We applied high-resolution mass-spectrometry-based proteomics to characterize 28 primary human hematopoietic cell populations in steady and activated states at a depth of >10,000 proteins in total. Protein copy numbers revealed a specialization of immune cells for ligand and receptor expression, thereby connecting distinct immune functions. By integrating total and secreted proteomes, we discovered fundamental intercellular communication structures and previously unknown connections between cell types. Our publicly accessible (http://www.immprot.org/) proteomic resource provides a framework for the orchestration of cellular interplay and a reference for altered communication associated with pathology.

* ***Rieckmann et al.*** *Nat Immunol. 2017, 5:583-593.*

L-arginine modulates T cell metabolism and enhances survival and anti-tumor activity

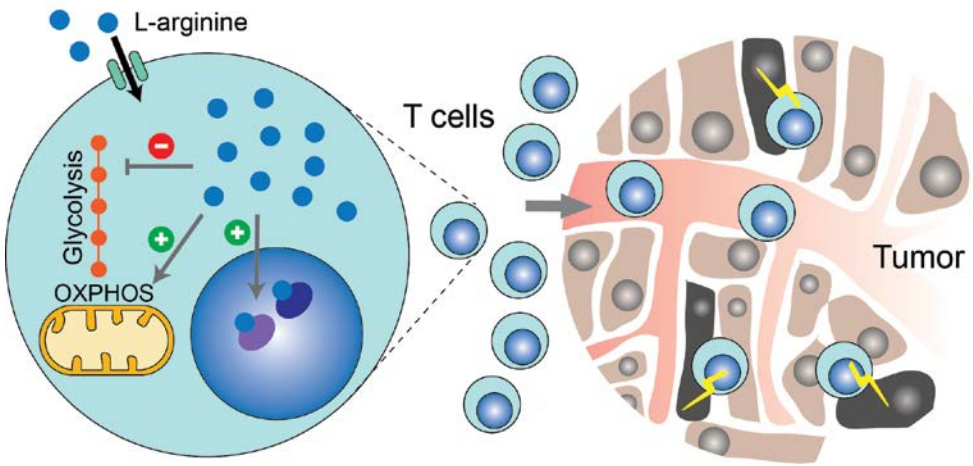
Roger Geiger, Tobias Wolf, Camilla Basso, Federica Sallusto, and Antonio Lanzavecchia

***Geiger et al.** **
Cell. 2016, 167:829-842.e13

Metabolic activity is intimately linked to T cell fate and function. Using high-resolution mass spectrometry, we generated dynamic metabolome and proteome profiles of human primary naive T cells following activation. We discovered critical changes in the arginine metabolism that led to a drop in intracellular L-arginine concentration. Elevating L-arginine levels induced global metabolic changes including a shift from glycolysis to oxidative phosphorylation in activated T cells and promoted the generation of central memory-like cells endowed with higher survival capacity and, in a mouse model, anti-tumor activity. Proteome-wide probing of structural alterations, validated by the analysis of knockout T cell clones, identified three transcriptional regulators (BAZ1B, PSIP1, and TSN) that sensed L-arginine levels and promoted T cell survival. Thus, intracellular L-arginine concentrations directly impact the metabolic fitness and survival capacity of T cells that are crucial for anti-tumor responses.

This work was done in collaboration with Paola Picotti and Nicola Zamboni at ETH Zurich, and with Mathias Mann and Felix Meissner at the Department of Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, Martinsried (DE).

Figure 5
Elevated intracellular L-arginine levels in T cells increase their anti-tumor functionality.



Translational activity and preparedness of naïve T cells

Tobias Wolf, Federica Sallusto, Antonio Lanzavecchia, and Roger Geiger

Naïve T cells circulate in the human body as small, quiescent cells with minimal metabolic activity. Yet, they need to be ready to rapidly respond to antigens and promptly execute a substantial activation program. To investigate the balance between "dormancy" and "readiness", we globally analyzed protein synthesis rates in naïve and activated T cells using a pulsed SILAC proteomics approach, and estimated absolute copy numbers of 7,800 proteins. In parallel, we sequenced transcripts and estimated copy numbers of mRNAs. We found that naïve T cells contain approximately 500 millions of proteins but only 14,000 mRNAs. The translational activity in naïve T cells was low and most proteins remained stable and were not synthesized within 48 hrs. However, a small subset of proteins was rapidly renewed including components of MHC-I and other cell surface receptors, components of the autophagy machinery and four transcriptional regulators whose murine homologs play a role in maintaining the naïve state of T cells. Despite little protein synthesis, naïve T cells contained enough ribosomal proteins to assemble 400,000 ribosomes. Several highly abundant mRNAs were hardly read off by ribosomes in naïve T cells but already six hours following activation these transcripts were read off at full capacity and the translational output per ribosome increased 8-fold. Thus, naïve T cells are equipped with large numbers of idle ribosomes and mRNAs that are rapidly engaged following stimulation to ramp up the activation program.

This work was done in collaboration with Mathias Mann and Felix Meissner, Max Planck Institute of Biochemistry, Martinsried (DE).

High-throughput TCR grafting for adoptive T cell therapies

Tobias Wolf, Elisabetta Loggi, Federica Sallusto, Antonio Lanzavecchia and Roger Geiger

Adoptive T cell transfer therapies have yielded significant benefit for a subset of patients with metastatic melanoma. For this therapy, tumor-infiltrating T cells from melanoma lesions are isolated and expanded *in vitro* and then infused back into patients in large numbers. The underlying assumption for using tumor-derived T cells is based on preclinical data showing that T cells bearing tumor-specific T cell receptors (TCRs) are strongly enriched in the tumor. However, unlike for melanoma patients, adoptive T cell transfer therapies have only yielded modest benefit for HCC patients. A likely reason is a problem in the *in vitro* T cell expansion procedure because tumor-reactive T cells isolated from HCC are severely exhausted, particularly the ones from chronic hepatitis B patients, and cannot respond to a proliferation stimulus. Consequently, non-specific T cells overgrow tumor-specific T cells in the cultures. To overcome this limitation and make adoptive T cell therapies accessible to HCC patients, we isolate exhausted T cells from tumors and directly graft their T cell receptor onto functional T cells. Using a microfluidics device, we encapsulate single tumor-derived T cells in picoliter droplets, in which all molecular biology steps are performed, including reverse transcription and PCR amplification of the TCR. As a result, we obtain a PCR product containing the sequence of the entire T cell receptor (alpha and beta chain linked together) from each T cell and ligate all PCR products *en bulk* into an expression vector. The resulting TCR libraries are transduced into engineered Jurkat T cells that are devoid of an endogenous TCR. Jurkat T cells that recognize tumor-antigens are then selected and their TCRs transferred onto functional primary T cells from patients to be used for therapy.

Collaborators: Andrew de Mello, ETH Zurich (CH) and Mathias Mann, Max Planck Institute for Biochemistry, Martinsried (DE)

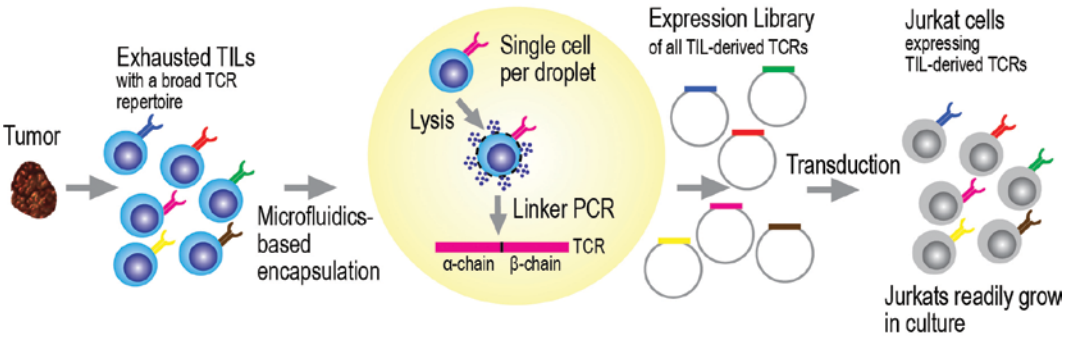


Figure 6
MIGOT: The TCR repertoire of exhausted T cells is mirrored on fast growing Jurkat cells.

Funding

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Antibody discovery and engineering

Bill & Melinda Gates Foundation
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Lectures and Seminars
2015

Ebola 2015 Conference: Challenging Ebola With a United Front
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Midwinter Conference 2015: Advances in Immunobiology
Seefeld in Tirol (AT) / 21.01.2015

25th Challenge in Virology Seminar
Gstaad-Saanen (CH) / 24.01.2015

The Human Vaccine Project Meeting
Lausanne (CH) / 06.02.2015

Keystone Symposium on Antibodies as Drugs: Immunological Scaffolds as Therapeutics
Banff (CA) / 11.02.2015

5th MilanMeetsImmunology (MMI) Meeting
Milan (IT) / 18.02.2015

Annual Congress SGAI / SSAI 2015
Basel (CH) / 13.03.2015

Keystone Symposium on HIV Vaccines: The Golden Anniversary of B Cells
Banff (CA) / 23.03.2015

Seminar at Pfizer Research Facilities
Cambridge (US) / 13.04.2015

Cold Spring Harbor Laboratory. Fundamental Immunology & Its Therapeutic Potential
Cold Spring Harbor (US) / 14.04.2015

Swiss Academy of Pharmaceutical Sciences 1st Forum "Trends in Pharmaceutical Sciences"
Bern (CH) / 20.04.2015

EMBO Gold Medallist Symposium 2015
Singapore (SG) / 11.05.2015

Immunology Workshop: Genetic control of immune cell activation – Implications for autoimmune diseases
Henningsvær (NO) / 21.08.2015

European Congress of Immunology Meeting 2015
Vienna (AT) / 08.09.2015

Systems Biology of Infection Symposium, 2nd Edition
Ascona (CH) / 09.09.2015

IBSA Foundation International Forum "Cancer immunology makes it to clinic: how cancer will be treated in the coming years"
Lugano (CH) / 26.09.2015

USI@EXPO Roundtable
Milan (IT) / 26.09.2015

Immune System for a Therapeutic Benefit Conference
Oropesa (ES) / 03.10.2015

Roundtable at Festival Bergamoscienza, 13th Edition
Bergamo (IT) / 04.10.2015

Herman Eisen Symposium
St. Louis (US) / 19.10.2015

XLI Annual Meeting of the Portuguese Society for Immunology
Braga (PT) / 28.10.2015

The Jenner Lecture 2015
London (UK) / 04.11.2015

Accademia Medica di Roma Seminar
Rome (IT) / 12.11.2015

Paul Erlich 2015 Symposium
Frankfurt (DE) / 23.11.2015

Immunotherapy@Brisbane 2015 Conference
Brisbane (AU) / 25.11.2015

Seminar at Singapore Immunology Network
Singapore (SG) / 27.11.2015

2016

Seminar at Karolinska Institutet
Stockholm (SE) / 22.01.2016

55th Midwinter Conference of Immunologists
Pacific Grove (US) / 26.01.2016

Distinguished Ludwig Lecture at UNIL (University of Lausanne)
Epalinges (CH) / 17.02.2016

SFB 1054 Symposium: Control and Plasticity of Cell-Fate Decisions in the Immune System
Munich (DE) / 03.03.2016

Gordon Research Conference on Antibody Biology & Engineering
Galveston (US) / 23.03.2016

Seminars on "New Frontiers in Monoclonal Antibody Therapy" at Collegio Borromeo
Pavia (IT) / 31.03.2016

Oxford Chemical Immunology Conference 2016
Oxford (UK) / 05.04.2016

Seminars on "New Frontiers in Monoclonal Antibody Therapy" at Collegio Borromeo
Pavia (IT) / 14.04.2016

HSM-2 Immunology Symposium "Immunotolerance: Moving towards Clinical Application"
Zurich (CH) / 17.05.2016

Keystone Symposium on B Cells at the Intersection of Innate and Adaptive Immunity
Stockholm (SE) / 02.06.2016

Seminar at the Weatherall Institute of Molecular Medicine
Oxford (UK) / 08.06.2016

Gordon Research Conference on Immunochemistry & Immunobiology: Immunity in Homeostasis, Disease and Therapy
Barga (IT) / 22.06.2016

Seminar at Bambino Gesù Ospedale Pediatrico
Rome (IT) / 05.07.2016

Seminar at Genentech, Inc.
South San Francisco (US) / 15.07.2016

Seminar at La Jolla Institute for Allergy & Immunology
La Jolla (US) / 19.07.2016

ICI 2016 Congress
Melbourne (AU) / 26.08.2016

Centre d'Immunologie de Marseille-Luminy (CIML) 40 Years Anniversary
Marseille (FR) / 15.09.2016

International Conference on Human & Translational Immunology
Rhodes (GR) / 18.09.2016

Immune Profiling in Health and Disease 2016 Nature Conference
Seattle (US) / 04.10.2016

Student-Organized Conference on "The Molecular Basis of Disease" at the Weizmann Institute of Science
Rehovot (IL) / 31.10.2016

1st Symposium on Emerging Viral Diseases "Understanding Emerging Viral Diseases and their Public Health Impact"
Geneva (CH) / 03.11.2016

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. It also led to the characterization of novel autophagic mechanisms ensuring removal from cells of toxic protein aggregates on destruction of the organelles that contain them. 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 ETH-Lausanne. In 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 and in 2017 he was elected member of the Faculty of 1000 for the Cell Biology Section.

Research Focus

The endoplasmic reticulum (ER) contains high concentrations of molecular chaperones and enzymes that assist maturation of newly synthesized polypeptides destined to the extracellular space, the plasma membrane and the organelles of the endocytic and secretory pathways. It also contains quality control factors that select folding-defective proteins for ER retention and/or ER-associated degradation (ERAD). Mutations, deletions and truncations in the polypeptide sequences may cause protein-misfolding diseases characterized by a "loss-of-function" upon degradation of the mutant protein or by a "gain-of-toxic-function" upon its aggregation/deposition. Pathogens hijack the machineries regulating protein biogenesis, quality control and transport for host invasion, genome replication and progeny production. Our long-standing interest is to understand the molecular mechanisms regulating chaperone-assisted protein folding and the quality control processes determining whether a polypeptide can be secreted, should be retained in the ER, or should be transported across the ER membrane for degradation. More recently, particular emphasis has been given to the characterization of responses (transcriptional or post translational) activated by cells expressing folding-defective polypeptides, to the mechanisms ensuring clearance of polymeric and aggregated proteins from the ER and to the study of select rare diseases such as α1-antitrypsin deficiency (ATD) and lysosomal storage diseases. 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: Timothy Bergmann, PhD student - Giorgia Brambilla Pisoni, PhD - Elisa Fasana, PhD - Ilaria Fregno, PhD student - Fiorenza Fumagalli, PhD student - Carmela Galli Molinari, MSc - Marisa Loi, PhD student - Tatiana Soldà, Msc.

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, Figure 1) 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, Hunter's diseases (Figure 2), α1-antitrypsin deficiency 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.

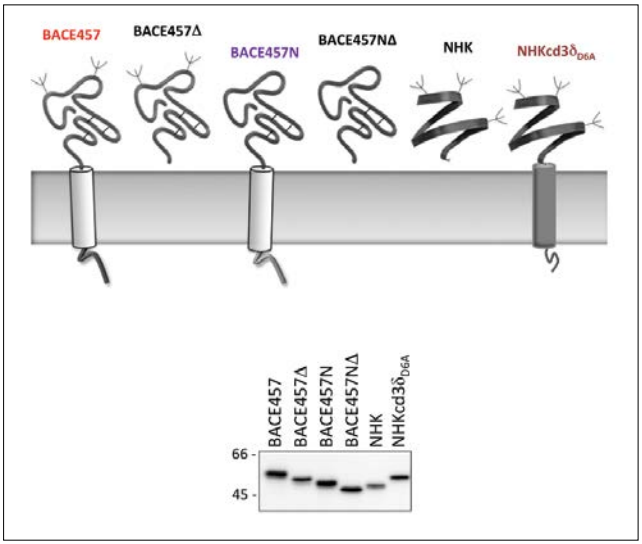

Figure 1
Selection of model substrates available in the lab.
BACE457 is a di-glycosylated type I membrane protein, BACE457Δ lacks the transmembrane domain, BACE457N lacks the protein-bound oligosaccharides, BACE457NΔ lacks transmembrane domain and oligosaccharides. NHK is a disease-causing, soluble polypeptide, NHKcd3δD6A is the same protein anchored at the membrane.

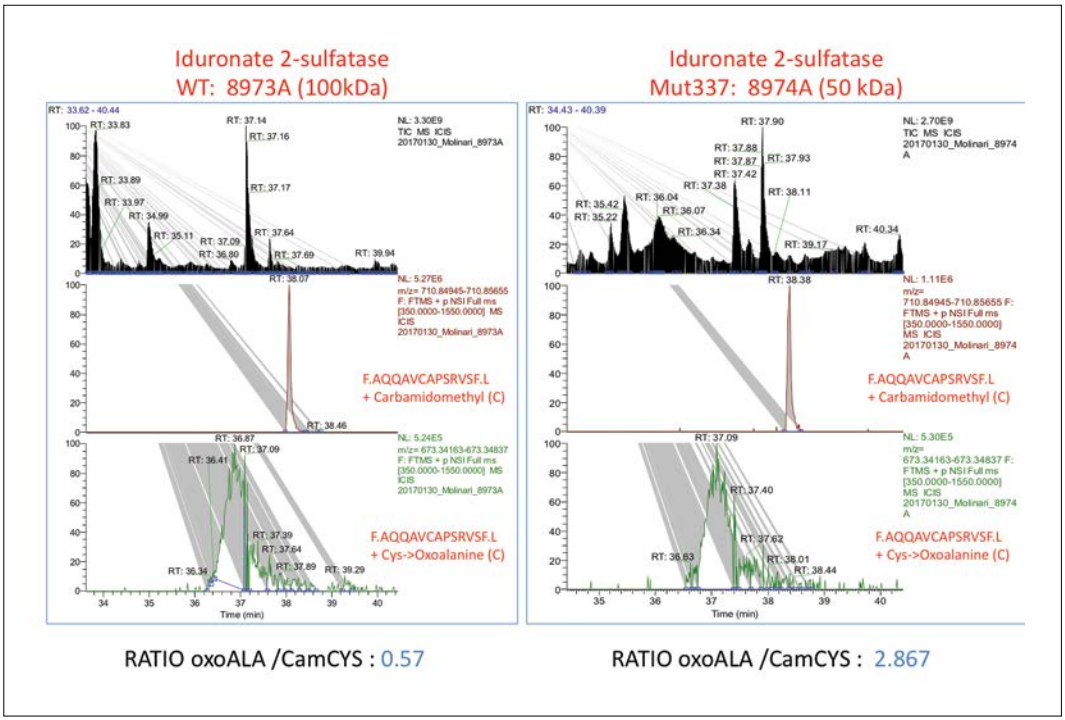

Figure 2
Hunter's disease
Comparison of activity-required modification of Cys84 in the wild type iduronate 2-sulfatase (links) and in a mutant form of the enzyme causing Hunter's disease (right).

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.

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

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

***Fregno and Molinari** **
Journal of Clinical Research on Rare Diseases. 2016; 1:40-42.

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 diseases 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. 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 α1ATcD6A). Ongoing work is focused on the identification of the components of this p97/UGGT1-mediated checkpoint.

Role of Membrane-Bound Oxidoreductases in Protein Biogenesis and Disposal

Giorgia Brambilla Pisoni, Concetta Guerra 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. 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 3). 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.

*** *Brambilla Pisoni et al***
Mol. Biol. Cell. 2015; 26:3390-3400.

*** *Tannous et al***
Semin. Cell Dev. Biol. 2015; 41:79-89.

*** *Molinari and Hebert***
Semin. Cell Dev. Biol. 2015; 41:70.

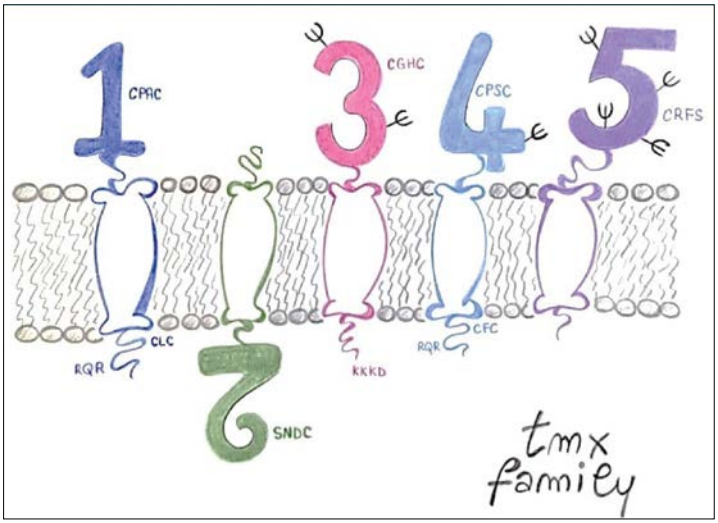

Figure 3
Hunter's disease
Topology of the 5 membrane-bound members of the protein disulfide isomerase superfamily. The topology and active site sequence of TMX1, TMX2, TMX3, TMX4 and TMX5 is shown. The protein-bound oligosaccharides in TMX3, TMX 4 and TMX 5 are shown in black.

Cellular Responses to Variations in ER Homeostasis and Protein Load

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

***Bergmann et al.** **
AIMS Biophysics. 2016; 3:456-478.

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 (Figure 4) 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, other proteins elicit UPR already at low dosage. Thus, the threshold for activation of transcriptional programs in response to increasing burden of misfolded protein 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.

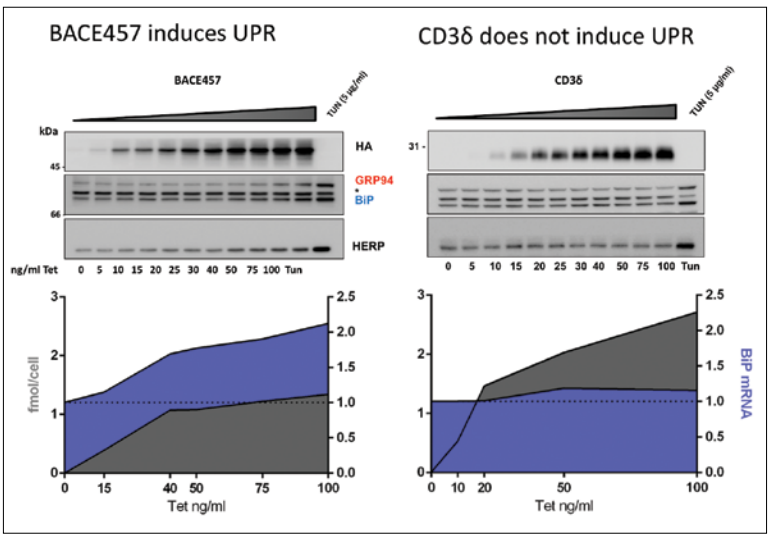

Figure 4
Inducible protein expression and cellular responses
Tet-induced expression of BACE457 (upper panel, left) and CD3δ (upper panel, right). BiP, GRP94 (middle panels) and HERP (lower panels) levels. Last lane, Tun-induced ER stress (5µg/ml). The graphs show variations of BACE457 (grey, left) and CD3δ (grey, right, fmol protein/cell) and BiP transcripts (blue) at doses of Tet ranging from 0 to 100 ng/ml cell culture media.

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

Ilaria Fregno, Timothy Jan Bergmann, Elisa Fasana, Carmela Galli, Tatiana Soldà 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. The disease-causing mutant protein used for initial studies will be the ATZ variant of α1 antitrypsin, whose accumulation is linked with hepatic failure in about 10% of the ATD patients.

Revealing Mechanisms Regulating Recovery from Transient ER Stress in Mammalian Cells

Fiorenza Fumagalli, Marisa Loi, Carmela Galli, Tatiana Soldà 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 ER membranes and chaperones produced during the stress phase is cleared from cells during the "recovery phase" occurring after ER stress resolution is the main subject of our study (Figure 5).

* ***Klionsky et al***
Autophagy. 2016; 12:1-222.

* ***Fumagalli et al***
Nature Cell Biol. 2016; 18:1173-1184.
- Highlights in Nature Reviews Molecular and Cellular Biology, 17
- Recommended by the Faculty of 1000

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Molecular and Cellular Oncology. 2017; 4:e1264351

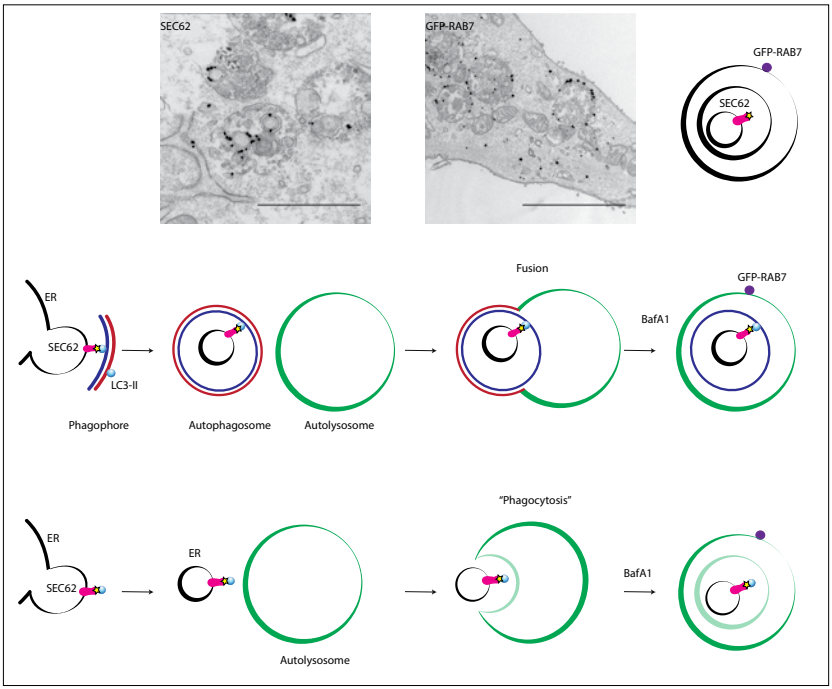

Figure 5
RecovER-phagy models
On recovery from ER stress, excess ER produced during the ER stress is delivered to autolysosomes for clearance. The immunoelectron microscopy images show gold-labeled SEC62 (left panel) within autolysosomes displaying GFP-RAB7 at the limiting membrane (right panel). The schematics show possible mechanisms of ER delivery to the autolysosomes (fusion mechanism, upper panel; phagocytic mechanism, lower panel).

Biogenesis and Fate of Disease-Causing Mutant Polypeptides

Ilaria Fregno, Timothy Jan Bergmann, Elisa Fasana, Alessandro Marazza

Mutations in the polypeptide chain may perturb the folding process. Misfolded polypeptides may be efficiently cleared from cells originating a so-called loss-of-function phenotype (i.e., the function of the mutant protein, which is cleared from cells, is missing). This is the case of conformational diseases such as cystic fibrosis, where mutant CFTR protein is rapidly removed from cells on intervention of the ubiquitin proteasome system (UPS). Alternatively, the mutant protein enters in aggregates that often cannot be properly handled by the UPS. In this case, autophagy may back-up and clear the misfolded proteins before it undergoes toxic deposition. If autophagy as well fails to remove the misfolded protein aggregates, the tissue may succumb on a gain-of-toxic-function phenotype. α1 antitrypsin deficiency (ATD) is a paradigmatic disease, studied in our lab, where some mutant proteins are efficiently cleared from cells generating a pure loss-of-function phenotype that compromises lung function (the NHK mutation) or a mixed loss- and gain-of-toxic-function phenotype where the lung disease is accompanied by liver failure caused by the toxic accumulation of polymeric mutant protein (the ATZ mutation) (Figure 6). The ATZ mutation enhances the polymerization propensity of the mutant protein. Surprisingly, however, only 10% of the ATZ patients eventually develop hepatic sufferance. Evidently, most of the patient's liver cells efficiently remove polymeric ATZ, a capacity that is lost in the rest of the patients (mostly children) that must undergo liver transplant. ATD is actually the main cause of genetic-related liver failure in children. ATD and lysosomal storage diseases (e.g., Hunter's disease) are among the rare diseases studied in our lab in an attempt to characterize common mechanistic features of conformational diseases deriving from expression of mutant gene products in the ER.

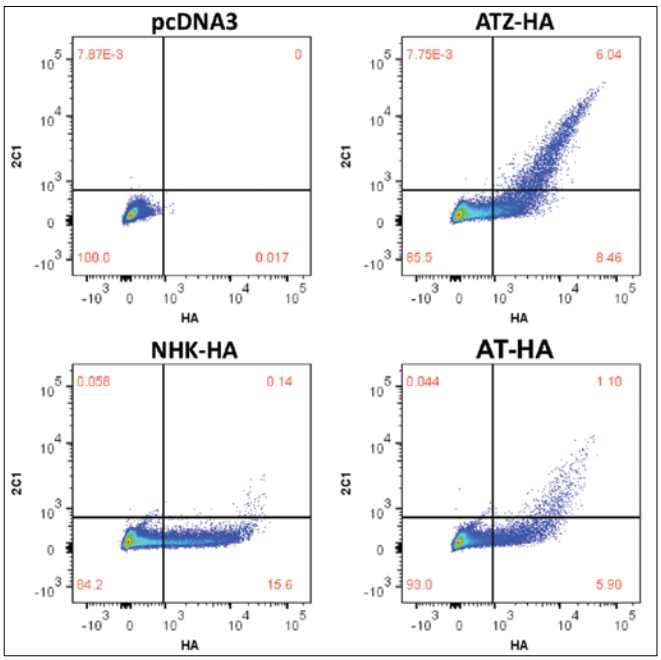

Figure 6
Analysis of polymerization propensity of wild type and mutant forms of α1-antitrypsin (AT).
pcDNA3 shows the immunoreactivity for total AT (x-axis, anti HA epitope) and polymeric AT (y-axis, anti 2C1 epitope). ATZ-HA, NHK-HA and AT-HA shows the same for the mutant ATZ and NHK forms and for the wt AT protein. The ATZ mutation substantially increases polymerogenicity.

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Funding

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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

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Concetta Guerra
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University of Padua (IT)

Alessandro Marazza
October-December 2016 (Stagiaire)

Publications

Role of SEC62 in ER maintenance: A link with ER stress tolerance in SEC62-overexpressing tumors?
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Lectures and Seminars

The ER and Redox Club Meeting
Division of labor between oxidoreductases: TMX1 preferentially acts upon membrane-bound Polypeptides
Venezia (IT) / 25-27.04.2015

11th International Calreticulin Workshop
A novel UGGT1 and p97-dependent checkpoint
New York (US) / 15-18.05.2015

FASEB Summer Research Conference "From Unfolded Proteins in the Endoplasmic Reticulum to Disease"
ERAD substrate-dependent regulation of ER-associated degradation
Saxtons River (US) / 14-19.06.2015

Meet the Expert
Rare Diseases: Alport's syndrome
Lugano (CH) / 04.05.2016

EMBO Meeting 2016, Structure and Function of the Endoplasmic Reticulum
Translocon component Sec62 acts in endoplasmic reticulum turnover during stress recovery
Girona (ES) 23-27.10.2016

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 at understanding 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. The main focus of her lab is the study of transcriptional and posttranscriptional mechanisms of regulation of gene expression in the activation and function of cells of the immune system, including the role of microRNAs as well as epigenetic modifications such as the methylation of genomic DNA.

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 autoimmune disorders such as multiple sclerosis. Epigenetic inheritance is usually independent from alterations in 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 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 autoimmune diseases.

Team

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Members: Michele Chirichella, Post-doc - Stefan Emming, PhD student - Cristina Leoni, PhD student - Sara Montagner, Post-doc - Lucia Vincenzetti, PhD student.

Epigenetic modifications in mast cells responses

Cristina Leoni, Sara Montagner and Silvia Monticelli

Mast cell activation is involved in the response to a variety of pathogens and allergens, making these cells an important effector type not only in innate immunity but also in allergic reactions and asthma. In addition, alterations in the number, localization, and reactivity of mast cells are typical features of systemic mastocytosis, a myeloproliferative disorder characterized by an increase in mast cell burden (Figure 1). Multiple genetic and epigenetic mechanisms can contribute to the onset and severity of all types of mast cell-related diseases. Methylation of the genomic DNA is an epigenetic process in which a methyl group is covalently linked to a cytosine base in the DNA, and such modification in our genome has a critical impact in the control of gene expression. Indeed, enzymes involved in catalyzing this process are implicated in the pathogenesis of a variety of diseases and in regulating the function of immune cells. The enzyme TET2 is responsible for the oxidation of 5-methylcytosine (5mC) in genomic DNA to 5-hydroxymethylcytosine (5hmC), and such modification contributes to gene transcriptional regulation and in some cases to tumorigenic transformation (Leoni, 2015).

We found that overall levels of genomic 5hmC and the activity of the TET enzymes were crucial in regulating mast cell differentiation from hematopoietic progenitors (Montagner, 2016), while appropriate patterns of DNA methylation and sufficient levels of DNA methyltransferase enzyme activity were critical to restrain mast cell inflammatory responses *in vivo* and in vitro, in response to both acute and chronic stimulation (Leoni, 2017). In other words, mast cells with a normal hydroxymethylation/ methylation pattern can differentiate and respond adequately to stimuli from the environment, while mast cells with an altered methylation pattern show abnormal proliferation and respond with exaggerated responses to normal stimuli, leading to unrestrained inflammation.

* ***Leoni C et al.***
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* ***Montagner S et al.***
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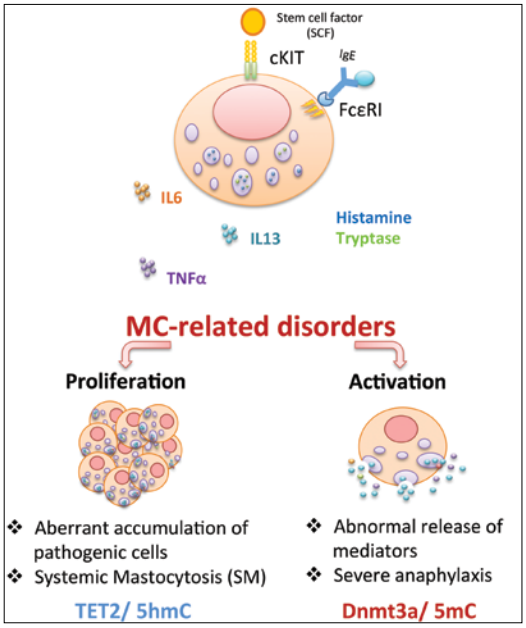

Figure 1
Mast cell responses in health and disease.
Mast cells act as important sentinels against danger signals in innate immunity, but alterations in the number, localization, and reactivity of mast cells are features of mast cell-related diseases such as systemic mastocytosis or allergy and asthma.

DNA methylation dynamics in the functional regulation of human T lymphocytes

Lucia Vincenzetti, Cristina Leoni 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.

Role of transcription factors in regulating human T cell activation and functions

Stefan Emming, Sara Montagner and Silvia Monticelli

Multiple Sclerosis (MS) is a chronic inflammatory disease with an autoimmune etiology mediated at least in part by $CD4^+$ T lymphocytes producing the pro-inflammatory cytokine Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF). Indeed, GM-CSF was shown to be necessary and sufficient to induce disease in several models of experimental autoimmunity. Levels of GM-CSF were also shown to be increased in patients with MS and to be associated with disease severity. We are investigating mechanisms that regulate the pathogenic potential of T lymphocytes, including the acquired ability to express high levels of GM-CSF. We identified optimal conditions to functionally separate primary human $CD4^+$ T lymphocytes based on their ability to produce high levels of inflammatory cytokines, such as GM-CSF, and we profiled both the transcriptome and miRnome of the cytokine-producing and non-producing populations. We successfully identified candidate genes and miRNAs specifically associated with either the cytokine-producing or non-producing phenotype, and we now initiated studies to understand the biological relevance of such candidates in primary human T lymphocytes from both healthy donors and people with MS.

MicroRNAs in the regulation of human T lymphocytes

Michele Chirichella, Stefan Emming and Silvia Monticelli

Mechanisms that regulate the threshold of T cell activation, as well as the magnitude and inflammatory potential of T cell responses are likely to be crucial in autoimmunity, and may become relevant therapeutic targets. Among the factors that can modulate T cell activation and responses, it is becoming increasingly clear that dysregulation of microRNA (miRNA) expression is involved in autoimmunity, implying that a detailed knowledge of miRNA-regulated gene expression networks is critical to gain understanding of normal and disease states. Utilizing a combination of cutting-edge cellular immunology techniques, in conjunction with the extensive investigation of the network of miRNA:mRNA interactions, we will ultimately determine the importance of miRNAs in regulating T cell pathogenicity in autoimmunity.

Funding

National Center of Competence in Research (NCCR) 'RNA & Disease'
Role of miRNAs in the regulation of human T cell activation and function in multiple sclerosis
2017-2020

Swiss National Science Foundation, Marie Heim-Vögtlin program
PMPDP3_164489 Post-doctoral Fellowship to Sara Montagner / 2016-2017

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

Kurt und Senta Herrmann-Stiftung
Role of miRNAs in the regulation of human T cell activation and in T cell lymphoma
2016

Ceresio Foundation
Fellowship contribution towards the salary of one PhD student / 2015-2016

Swiss Multiple Sclerosis Society
Understanding the molecular links between T cell pathogenicity and GM-CSF production in multiple sclerosis / 2015

San Salvatore Foundation for Cancer Research
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Lectures and Seminars

EpiGeneSwiss meeting
DNA methylation and hydroxymethylation regulate mast cell differentiation and function
Weggis (CH) / 29-31.5.2017

University of Oxford, Sir William Dunn School of Pathology
DNA methylation restrains effector functions in immune cells
Oxford (UK) / 10.04.2017

Workshop "Noncoding RNAs in the immune system"
MicroRNA-mediated regulation of T cell functions
Milan (IT) / 19-20.07.2016

EFIS course "Autoimmunity: Basic and Clinical"
MicroRNAs in immunity and autoimmunity
Tartu (EE) / 6-8.06.2016

NCCR RNA & Disease Retreat
MicroRNA-mediated regulation of immune cell functions
Kandersteg (CH) / 19-21.01.2016

University of Lausanne, Department of Biochemistry
MicroRNA-mediated regulation of immune cell functions
Lausanne (CH) / 04.12.2015

Keystone Symposium 'MicroRNAs and Noncoding RNAs in Cancer'
MicroRNA-Mediated Regulation of T Lymphocyte Activation
Keystone, CO (US) / 7-12.06.2015

Federica Sallusto

Cellular Immunology

Federica Sallusto

Federica Sallusto received the degree of Doctor in Biology from the University of Rome in 1988, and performed postdoctoral work at the Istituto Superiore di Sanità in Rome and at the Basel Institute for Immunology, where she was a member from 1997 to 2000. Since 2000 she is Group leader of the Cellular Immunology Laboratory at the IRB where she has also established the Center of Medical Immunology in 2016. In February 2017, she was appointed Professor of Medical Immunology at the ETH Zurich. Among her original contributions are the development of a method to culture human dendritic cells, the discovery that human Th1, Th2 and Th17 cells express distinct sets of chemokine receptors, the definition of central and effector memory T cell subsets and of skin-homing Th22 cells and the definition of two distinct types of Th17 cells. In the mouse system, her work has shown that NK cells, T helper cells, and cytotoxic T cells can migrate to inflamed lymph nodes, where they profoundly modulate T cell responses, and that encephalitogenic Th17 cells use CCR6 to enter the CNS through the choroid plexus. 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 Study of Neurodegenerative Diseases in 2010. She was elected member of the German Academy of Sciences Leopoldina in 2009 and of EMBO in 2011. From March 2013 to March 2015 she was president of the Swiss Society for Allergology and Immunology.

Research Focus

The focus of our laboratory is the analysis of the immune response in humans using novel high throughput cell-based assays complemented with powerful analytical technologies, such as next generation sequencing, single cell transcriptomics, metabolomics and proteomics. With our studies, we are defining the signals through which cells of the innate immune system, such as dendritic cells and monocytes, determine the differentiation, proliferation and long-term survival of cells of the adaptive immune system. These studies aim to address fundamental questions related to how the immune system can protect us against different classes of microbial pathogens, such as viruses, or bacteria, and to provide insights for the design of new and more effective vaccine strategies. More recently, we are conducting studies to understand why in patients with chronic or disseminated infections, including children with rare primary immunodeficiencies caused by genetic disorders, the immune system fails to protect the host. By applying the same experimental approach, we perform studies to understand how some individuals mount immune responses against not harmful environmental antigens or self-antigens, which cause allergy and autoimmunity. In this context, we are continuing our studies, in patients suffering from neurological disorders, including multiple sclerosis and, more recently, narcolepsy, in collaboration with university hospitals in Zurich, Bern and Genova, and the Neurocenter of Southern Switzerland. Finally, we are developing new tools to advance the highly active and exciting field of cancer immunotherapy.

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Former Members: Dominik Aschenbrenner, PhD - Silvia Preite, PhD - Tomasz Wypich, PhD
Visiting scientists: Cristina Gagliardi, MD - Janet Markle, PhD - Mariana Pereira Pinho

TCR stimulation triggers distinct late transcriptional programs that characterize regulatory and pro-inflammatory human Th17 cell subsets

Dominik Aschenbrenner, Mathilde Foglierini, David Jarrossay, Samuele Notarbartolo, and Federica Sallusto

Effector $CD4^+$ T helper cells play an important role in host defence but also mediate inflammatory diseases, such as autoimmunity or allergy. The function of these cells critically depends on the range of cytokines produced and on the balance between pro- and anti-inflammatory cytokines. We found that production of the anti-inflammatory cytokine IL-10 is restricted to a subset of human memory Th17, Th1 and Th2 cells and occurs at late time points after activation. Using wide gene expression analysis and chromatin immunoprecipitation assays, we identified genes that are differentially expressed on IL-10^+ and IL-10^- Th17 cells and investigated the mechanisms by which these genes are transcriptionally regulated. Genes highly expressed in IL-10^- Th17 cells included *IL23R, IFNG, CSF2, IL1R1, IL12RB2, IL2, IL2RA* and *IL22*, which have been associated with "pathogenic" mouse Th17 cells. Genes highly expressed in IL-10^+ Th17 cells included *IL10, CTLA4, IKZF3, PDCD1, TGFB1, LRRC32, BACH2, LGMN* and *P2RX7*, which have been associated with "non-pathogenic" mouse Th17 cells. Late-activated human IL-10+ Th17 cells also upregulated genes characteristic of tissue resident T cells, suggesting that after antigenic stimulation, these cells may persist in peripheral tissues to confer long-term protection while avoiding over-inflammation. IL-10^+ Th17 cells were characterized by a high expression of the transcription factor c-MAF. Notably, c-MAF was found to bind about half of the genes differentially expressed in IL-10^+ vs. IL-10^- Th17 cells. Furthermore, c-MAF extensively bound *bona fide* enhancers in activated IL-10^+ Th17 cells and its binding was particularly enriched in proximity of immune response genes. Finally, using molecular approaches to ectopically express or silence c-MAF, we demonstrated that c-MAF not only regulates *IL10* expression but it is also necessary and partially sufficient to promote a broad transcriptional program favouring auto-regulatory over pro-inflammatory Th17 cells (Figure 1). Thus, c-MAF represents a relevant factor discriminating between pathogenic and non-pathogenic T_H17 cells.

Collaborators: Vijay K. Kuchroo and Howard L. Weyner, Harvard Medical School, Boston (US)

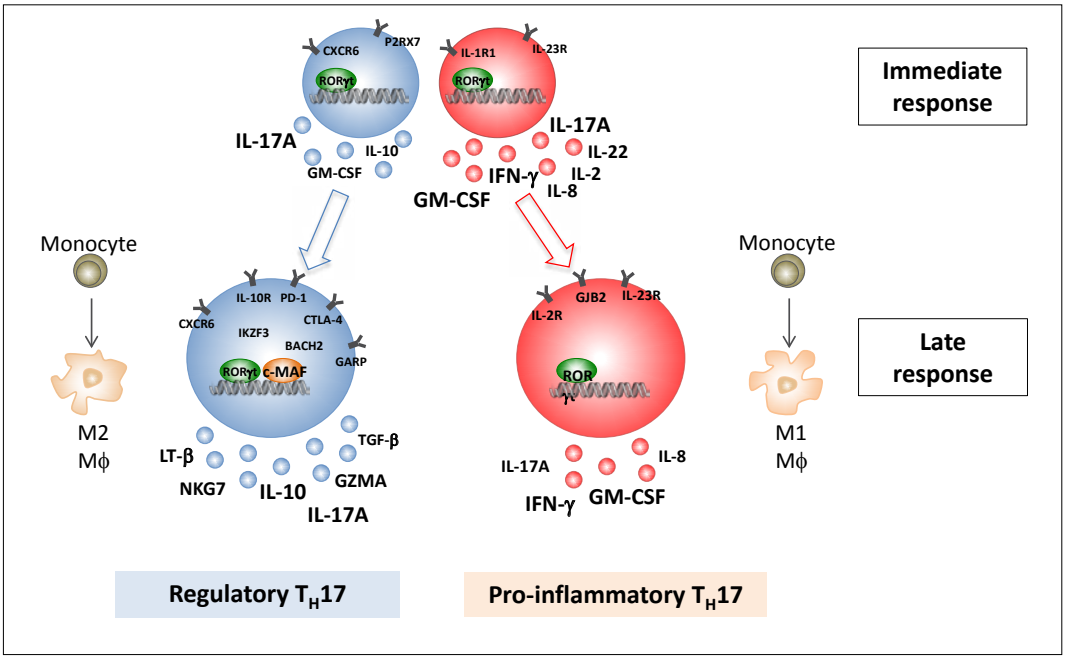

Figure 1
c-MAF represents a relevant factor discriminating between regulatory and pro-inflammatory human Th17 cells

From antigen presentation to antigen recognition: dissecting the human T helper cell repertoire to Influenza virus

Antonino Cassotta, Roger Geiger, Marco Benevento, Philipp Paparoditis, Mathilde Foglierini, Antonio Lanzavecchia, and Federica Sallusto

Influenza viruses represent a public health concern due to their pandemic potential and to the sporadic spread of highly pathogenic strains from zoonotic hosts to humans. Several recent studies on the clonal composition of the human antibody repertoire against hemagglutinin (HA) – a main target of neutralizing antibodies against Influenza virus – have revealed that broadly neutralizing antibodies against conserved epitopes in the HA stem region can develop in the course of an immune response to infection or vaccination. Surprisingly, the repertoire of human CD4$^+$ T helper (Th) cells against HA remains poorly defined, in spite of the fact that these cells play an important role in the induction of the antibody and cytotoxic CD8$^+$ T cell responses. In this study, we set out to understand the role of antigen presentation in shaping the antigen-specific human Th cell repertoire and its contribution in the development of immunological memory following vaccination. By combining antigenic stimulation of naïve and memory T cell libraries, T cell cloning, epitope mapping and TCR sequencing, we provide a comprehensive description of the clonal composition of the human Th cell repertoire against HA. Furthermore, using mass spectrometry-based approach we define the MHC-II immunopeptidome of monocytes-derived dendritic cells and polyclonal and HA-specific B cells

pulsed with HA or whole Influenza virus. This study can shed new light on the mechanisms underlying T cell repertoire selection, immunodominance, and formation of immunological memory, and could have important implications for vaccine design and prediction of immunization outcome.

Collaborator: David JM Lewis, University of Surrey (UK)

Phenotype and specificity of T cells in primary human cytomegalovirus infection in pregnancy and their association with protection from vertical transmission

Federico Mele, David Jarrossay, Federica Sallusto, Daniele Lilleri

Congenital human cytomegalovirus (HCMV) infection is the major cause of birth defects. A precise definition of the HCMV-specific T cell response may help defining reliable correlates of immune protection in pregnancy. In collaboration with the group of Giuseppe Gerna at the Fondazione IRCCS Polilclinico San Matteo, Pavia, we used a high throughput method to define the frequency of $CD4^+$ and $CD8^+$ T cells specific for four relevant HCMV proteins in the naïve compartment of seronegative subjects and in the effector and memory compartments of subjects with primary or remote HCMV infection. We found that the naïve repertoire contains comparable frequencies of T cells reactive against structural (pp65, gB and gHgLpUL128L) and non-structural (IE-1) proteins. However, upon *in vivo* infection, the majority of effector and memory $CD4^+$ and $CD8^+$ T cells were found to recognize gB and IE-1, respectively, as well as pp65. The pattern of T cell reactivity was comparable at early and late stages of infection and in pregnant women with primary HCMV infection transmitting and non-transmitting the virus to the fetus. At early stages of infection, about 50% of HCMV-reactive $CD4^+$ T cells were "short-term" IL-7Rneg effector cells. At 6-12 months after infection, the frequency of these cells decreased to become negligible in remote infections, concomitant with an increase in the frequency of "long-term" IL-7Rpos memory cells. In contrast, 80-90% of HCMV-specific $CD8^+$ T cells were short-term effectors up to 12 months after primary infection, decreasing to 30% in remote infection. Interestingly, a higher frequency of HCMV-specific $CD4^+$ T cells with a long-term IL-7Rpos memory phenotype was observed in non-transmitting women compared to transmitting women. These findings indicate that immunodominance in HCMV infection is not predetermined in the naïve compartment but is the result of virus-host interaction and suggest that a prompt control of HCMV infection in pregnancy is associated with a rapid development of HCMV-specific long-term memory $CD4^+$ T cells and a low risk of virus transmission to the fetus.

This project is a collaboration between IRB and Fondazione IRCCS Policlinico San Matteo, Pavia, (IT).

Specificity and cross-reactivity of T cells elicited by Zika virus infection

Antonino Cassotta, Federico Mele, and Federica Sallusto

***Stettler K et al.** **
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Zika virus (ZIKV) and Dengue viruses (DENV) belong Flaviruses family and represent a public health emergency. In the case of DENV, a primary infection protects from reinfection with the same serotype, but represents a risk factor for the development of haemorrhagic fever upon reinfection with a different serotype, possibly due to the presence antibodies that fail to neutralize the incoming virus but instead enhance its capture by Fc receptor-expressing cells, leading to enhanced viral replication and activation of cross-reactive memory T cells. Whether individuals with antibodies induced by previous DENV infections can develop a more severe ZIKV infection or have higher risk of fetal transmission is unknown. Similarly, it is unclear whether ZIKV antibodies may impact subsequent DENV infection. Therefore, it is important to dissect the level of cross-reactive immunity at the B- and T-cell level in response to DENV and ZIKV infection. In a collaborative effort with the group of Davide Corti at Humabs BioMed, we found that monoclonal antibodies (mAbs) isolated from DENV or ZIKV convalescent patients that recognize NS1 or the domain III (DIII) of the E protein are mainly DENV or ZIKV specific. In contrast, the large of majority of the mAbs specific for the domain I and II (DI/II) of the E protein fully cross-react between DENV and ZIKV and, while poorly neutralizing, they potently enhance DENV and ZIKV infection. Given the role of T cells in antibody production and in immunopathology, we also investigated the specificity and cross-reactivity of CD4+ memory T cells from the same donors using the T cell library method. NS1-specific and E-specific memory CD4+ T cells were primarily present in a CXCR3+ Th1 compartment and, with a few exceptions, were specific for either ZIKV or DENV, even in donors with ZIKV infection and pre-exposed to DENV. These data indicate variable levels of cross-reactivity in the humoral and cellular response to DENV and ZIKV.

This project is a collaboration between IRB and Humabs BioMed, Bellinzona (CH).

Clonotypic analysis of the T cell response to Mycobacteria in subjects with latent tuberculosis infection or BCG vaccination

Federico Mele, Daniel Hoces, Sandra Jovic, and Federica Sallusto

The long-standing relationship between the human host and *Mycobacterium tuberculosis* (MTB) is complex. A profound understanding of the protective immune responses that develop in some individuals with latent tuberculosis infection (LTBI) or previous vaccination with Bacillus Calmette-Guérin (BCG) is instrumental to develop an effective vaccine and better treatments. Our previous work identified a subset of $CXCR3^+$ Th1 cells (defined as Th1*) induced by MTB and that can be distinguished from classic $CXCR3^+$ Th1 cells based on the expression of the chemokine receptor CCR6 (Figure 2). Previous work has also shown that non-exposed healthy non-BCG vaccinated individuals (HCs) have memory T cells cross-reactive against MTB sequences, suggesting environmental exposure to nontuberculous mycobacteria (NTMs) may impact on the ensuing response to MTB. In collaboration with Alessandro Sette and Bjoern Peters at the La Jolla Institute for Allergy and Immunology, we found that T cells elicited by MTB/NTM cross-reactive epitopes in HCs are found mainly in a $CCR6^+CXCR3^+$ memory subset, similar to findings in LTBI individuals. Thus, both MTB and NTM appear to elicit a phenotypically similar T cell response. Furthermore, T cells reactive to MTB/NTM-conserved epitopes responded to naturally processed epitopes from MTB and NTMs, whereas T cells

reactive to MTB-specific epitopes responded only to MTB. NTM-specific epitopes that elicit T cells that recognize NTMs but not MTB were identified. These epitopes can be used to characterize T cell responses to NTMs, eliminating the confounding factor of MTB cross-recognition and providing insights into vaccine design. We are now using deep sequencing to study the T-cell receptor repertoire of antigen-specific $CD4^+$ memory T cells against selected epitopes of MTB and NTM in donors with LTBI or BCG vaccination. We found that the antigen-specific response in both groups of donors had a low diversity, with a limited number of clonotypes highly expanded. In LTBI donors, a variable number of clonotypes were found in both MTB- and NTM-responding T cell populations; these shared clonotypes showed a different pattern of expansion in different donors. Finally, no public MTB- or NTM-specific T-cell receptors or consensus sequences were found among the studied donors. Our results indicate that although the immune response against selected epitopes of MTB and NTM is mostly driven by oligoclonal Th1* cells, the expanded antigen-specific clonotypes are different among individuals, which may explain the variable degree of protective immune response described in LTBI and BCG-vaccinated populations.

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This project is a collaboration between IRB and La Jolla Institute for Allergy and Immunology, La Jolla (US).

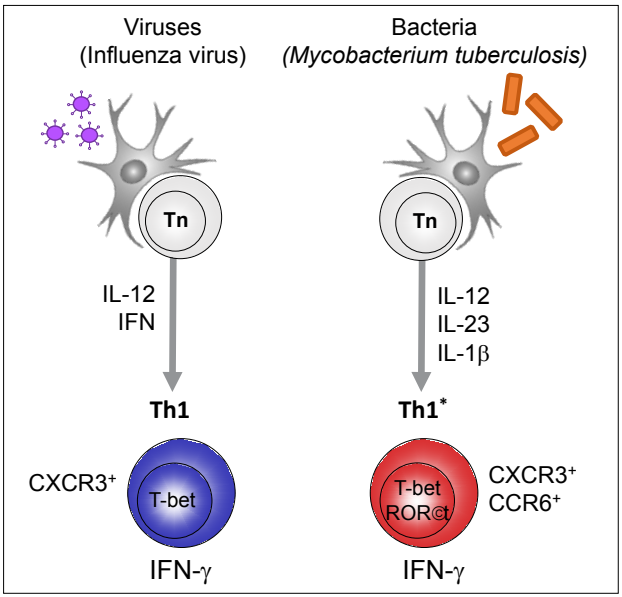

Figure 2
Two types of human Th1 cells preferentially elicited by viruses or bacteria.

The human immune T cell response to commensals

Jérémie Goldstein, Daniela Latorre, Antonino Cassotta, Cristina Gagliardi, 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+ Th1/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. The class and cross-reactivity of T cells induced in response to the Gram-negative bacterium *Klebsiella pneumonie*, an important pathogen in nosocomial infections, is also currently investigated.

Collaborator: Istituto Cantonale di Microbiologia, Bellinzona (CH)

Class and specificity of T cells against *C. albicans* in normal and pathological conditions

Corinne De Gregorio, Federico Mele, Roger Geiger, Marco Benevento, Sandra Jovic, and Federica Sallusto

$CD4^+$ T helper cells are crucial players in the adaptive immune response against microbial pathogens and can be divided in different Th subsets characterized by distinct functional properties, such as cytokine production and chemokine receptor expression. We developed a high throughput cell based assay that, combined with sorting of T cell subsets and *in vitro* T cell priming assays, can provide new insights on the class and specificity of the human T cell response to pathogens. We particularly focused on the opportunistic fungal pathogen *Candida albicans* and 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, unraveling an unexpected degree of intraclonal functional heterogeneity of the human T cell response. Surprisingly, in spite of the well-known role of Th cells in protection against fungal diseases, specific *C. albicans* antigens responsible for their activation remain poorly defined. We are using a mass spectrometry-based approach to define the MHC-II immunopeptidome of monocytes-derived dendritic cells pulsed with *C. albicans*. Moreover, to understand the role of different T cell subsets in host protection, we are studying, in collaboration with the group of Jean Laurent Casanova at the Rockefeller University and Anne Puel at the Imagine Institute, the repertoire (class, frequency, and clonotypic composition) of *C. albicans*-specific T cells in patients with primary immunodeficiencies suffering from chronic mucocutaneous candidiasis (CMC). Patients with STAT1 gain-of-function mutations (STAT1-GOF) showed 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. Currently, we are analyzing patients with STAT3 loss-of-function

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

Okada S, et al. ***
Science 2015, 349:606-613.

mutations (STAT3-LOF) and patients with other primary immunodeficiencies (IL-17RA-deficiency, IL23R-deficiency). These studies are expected to improve our knowledge about disease pathogenesis and may be useful for the design of novel immunotherapeutic strategies.

This project is a collaboration between the IRB, the Rockefeller University, New York (US), and the Imagine Institute, Paris (FR).

Innate and adaptive immune response to *C. albicans*

Roberta Marzi, Camilla Basso, 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. 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.

Collaborator: Burkhard Becher, University of Zurich (CH).

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

Sara Natali, Tess M. Brodie, 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. TCR sequencing of HDM-reactive $CRTh2^{+}$ and $CCR10^{+}$ T cells indicates a different TCR repertoire, suggesting that these cells originate from distinct precurosrs possible primed in

different anatomical locations. Finally, we found that IL-9 production was flexible in CRTh2$^+$ cells and it was induced by strong co-stimulatory signals and TGF-β. In conclusion, our study revealed the heterogeneous and multifunctional phenotype of allergen-reactive T lymphocytes.

Collaborators: Alessandro Sette and Cecilia S. Lindestam Arlehamn, La Jolla Institute for Allergology and Immunology, La Jolla (US).

The autoimmune basis of narcolepsy

Daniela Latorre, Eric Armentani, Mathilde Foglierini, Federico Mele, Antonino Cassotta, and Federica Sallusto

Narcolepsy with cataplexy is a hypothalamic disorder caused by the selective loss of neuronal cells of the posterior hypothalamus that produce the neuropeptide hypocretin (HCRT). As a result, HCRT-1 levels in cerebrospinal fluid (CSF) are low or undetectable in >95% of narcolepsy-cataplexy patients. Accumulating lines of evidence, including a strong association with the HLA-DQB1*06:02 haplotype, support the notion that narcolepsy is an immune-mediated disorder that manifests in genetically predisposed individuals upon exposure to environmental factors. The involved cellular and/or humoral mechanisms are still unclear. To gain insights into the pathogenic mechanisms of narcolepsy we established a collaboration with Claudio Bassetti (University Hospital Bern), Mauro Monconi (Neurocenter of Southern Switzerland), Ramin Khatami (Barmelweid Clinic) and Mehdi Tafti (University of Lausanne). To characterize memory T cells from narcolepsy patients we combined antigenic stimulation, T cell cloning, and TCR deep sequencing. Autoreactive CD4$^+$ T cell clones specific for HCRT and other self-antigens expressed by HCRT-producing neurons were isolated from most patients, including those lacking the HLA-DQB1*06:02 allele, but were not found in healthy HLA-DQB1*06:02 donors. The T cell response was polyclonal, restricted by HLA-DR and HLA-DQ molecules and directed against multiple epitopes. Interestingly, most autoreactive clones recognized exogenous peptides but failed to respond to whole proteins, suggesting that the epitopes recognized are generated through extracellular processing. TCR sequencing on paired blood and CSF samples identified shared clonotypes and demonstrate the presence of a few autoreactive T cell clones in the CSF. In addition, public clonotypes (shared by 3 and more individuals) were significantly more expanded in narcolepsy patients as compared to controls. Collectively, our data demonstrate the existence in narcolepsy patients of expanded autoreactive T cells targeting different neuronal antigens and suggest a mechanism for escape from tolerance through unconventional antigen processing.

This project is a collaboration between the IRB, the University Hospital Bern, the Neurocenter of Southern Switzerland, the Barmelweid Clinic, and the University of Lausanne (CH).

Analysis of the tumor-antigen specific T cell repertoire in healthy donors using T cell libraries

Mariana Pereira Pinho, Roger Geiger, Tobias Wolf, Jérémie Goldstein, and Federica Sallusto

During tumorigenesis, transformed cells express tumor-associated antigens (TAAs), which can be recognized by the adaptive immunity. TAA-specific T cells can be detected in cancer patients and also in healthy individuals, but their broad specificity patterns, frequency and functional phenotype are still unknown. Using the T cell library method, we set out to determine frequency and phenotype of T cells specific against pool of peptides containing mutated portions of proteins found in a patient with hepatocellular carcinoma. Libraries of blood $CD4^+$ T cells from two healthy donors were screened using monocytes or B cells as APCs, and one donor showed memory T cell lines that were reactive against the tumor peptide pool. The calculated precursor frequency of CD4 total memory T cells specific for the tumor peptides was of 5 specific T cells/million on the healthy donor, compared to 21 T cells/million found in the library of the patient used to design the peptide. Since only 400,000 T cells could be screened in the library, and tumor peptide-specific T cells showed to be rare, we developed a way to determine the number of specific T cells by using direct ex vivo stimulation of all available T cells. By using this method and screening more than 5 million T cells of a healthy donor, we could find naïve (2.1 cells/million) and memory (0.8 cells/million) $CD4^+$ T cells specific for the tumor peptides. In conclusion, we determined that healthy donors have rare $CD4^+$ T cells specific for tumor neoantigens in the blood, which were found with a higher frequency in a patient bearing a tumor expressing the mutated antigens.

Collaborator: José Alexandre Marzagão Barbuto, University of São Paulo (BR).

The Tfh-like transition during human Th1 differentiation

Mengyun Hu and Federica Sallusto

T follicular helper cells (Tfh) are a distinct subset of $CD4^+$ T cells specialized to provide help to B cells for antibody production and affinity maturation. Tfh cells are characterized by the expression of the chemokine receptor CXCR5, the markers PD-1 and ICOS, and the transcription factor Bcl-6. The differentiation of human Tfh and their relationships with other Th cell subsets remain unclear and have been the subject of intense investigation in recent years. In this study, we primed CD4 naïve T cells in MLR model (Mixed Lymphocytes Reaction) to generate a Th1 driven immune response. We found that activated T cells diverged into $CXCR5^+$ and $CXCR5^-$ populations as early as the first cell division. The phenotypes of these two subsets were also different. $CXCR5^-$ cells developed typical Th1 phenotype, expressed mainly IFN-γ and the transcription factor T-bet. CXCR5+ cells had phenotypic characteristics of both Th1 and Tfh cells, including the expression of Bcl-6 and T-bet and production of IFN-γ and IL-21. However, this Tfh-Th1 cell like phenotype was transient. TCR sequencing of the two populations revealed a low extent of clonotype sharing. Studies are ongoing to define the dynamic relationship between Tfh and Th1 cells during differentiation.

Somatic mutations and affinity maturation are impaired by excessive numbers of T follicular helper cells and restored by Treg cells or memory T cells

Silvia Preite, Mathilde Foglierini, Camilla Basso, Blanca Fernandez Rodriguez, Antonio Lanzavecchia and Federica Sallusto

Preite S, et al. ***
Eur J Immunol, 2015, 45:3010-3021.

We previously reported that *Cd3e*-deficient mice adoptively transferred with $CD4^+$ T cells generate high numbers of T follicular helper (Tfh) cells, which go on to induce a strong B-cell and germinal center (GC) reaction. Here we show that in this system, GC B cells display an altered distribution between the dark and light zones, and express low levels of activation-induced cytidine deaminase (AID). Furthermore, GC B cells from $Cd3e^{-/-}$ mice accumulate fewer somatic mutations as compared with GC B cells from wild type (WT) mice, and exhibit impaired affinity maturation and reduced differentiation into long-lived plasma cells. Reconstitution of $Cd3e^{-/-}$ mice with regulatory T cells restored Tfh-cell numbers, GC B-cell numbers and B-cell distribution within dark and light zones, and the rate of antibody somatic mutations. Tfh-cell numbers and GC B-cell numbers and dynamics were also restored by pre-reconstitution of $Cd3e^{-/-}$ mice with $CXCR5^{-/-}$ Treg cells or non-regulatory, memory $CD4^+$ T cells. Taken together, these findings underline the importance of a quantitatively regulated Tfh-cell response for an efficient and long-lasting serological response.

B cells can initiate and maintain T helper cell responses in a mouse model of asthma via BCR independent antigen presentation

Tomasz Piotr Wypych, Roberta Marzi, and Federica Sallusto

The importance of B lymphocytes to present antigens for antibody production is well documented. In contrast, very little is known about their capacity to influence $CD4^+$ T cell activation during primary or secondary response to allergens. In this study, we used mouse models of asthma to investigate the role of B cells as antigen presenting cells in propagation and initiation of T helper cell responses. Mice were immunized via intranasal route with house dust mite extract (HDM) derived from Dermatophagoides pteronyssinus. To investigate the importance of B cells in maintenance of allergic response, B cells were depleted in HDM-sensitized animals. To investigate the role of B cells in T cell priming, B cells were depleted before HDM sensitization; furthermore, HDM sensitization was performed in mice with MHC-II expression restricted to the B cell lineage. We found that lung B cells serve as potent antigen presenting cells ex-vivo and restimulate *in vivo* primed HDM specific T helper cells. HDM antigens were taken up by B cells independently of the B cell receptor specificity, indicating that HDM uptake and antigen presentation to $CD4^+$ T cells is not restricted to rare antigen-specific B cells. B cell depletion before HDM challenge in HDM sensitized mice resulted in a dramatic reduction of allergic response, indicating the role of B cells in amplification of Th2 responses. In contrast, HDM sensitization of mice in which MHC-II expression was restricted to B cells revealed the inability of these cells to initiate Th2 responses, but highlighted their unexpected role in priming Th1 and Th17 responses. Collectively, these data reveal new mechanisms leading to initiation and exacerbation of allergic response that may have implications for designing new therapeutic strategies to combat house dust mite allergy.

Collaborator: Gregory F Wu, Washington University, St. Louis (US).

Activin-A co-opts IRF4 and AhR signaling to induce human regulatory T cells that restrain asthmatic responses

Sofia Tousa, Tess M Brodie, and Federica Sallusto

Type 1 regulatory T (Tr1) cells play a pivotal role in restraining human T-cell responses toward environmental allergens and protecting against allergic diseases. Still, the precise molecular cues that underlie their transcriptional and functional specification remain elusive. In collaboration with the team led by Georgina Xanthou at the Biomedical Research Foundation of the Academy of Athens, we found that the cytokine activin-A instructs the generation of CD4+ T cells that express the Tr1-cell-associated molecules IL-10, ICOS, LAG-3, and CD49b, and exert strongly suppressive functions toward allergic responses induced by naive and *in vivo*-primed human Th2 cells. Moreover, mechanistic studies reveal that activin-A signaling induces the activation of the transcription factor IRF4, which, along with the environmental sensor aryl hydrocarbon receptor (AhR), forms a multipartite transcriptional complex that binds to IL-10 and ICOS promoter elements and controls gene expression in human CD4+ T cells. IRF4 silencing abrogates activin-A-driven *IL10* and *ICOS* up-regulation and impairs the suppressive functions of human activin-A-induced Tr1-like (act-A-iTr1) cells. Importantly, using a humanized mouse model of allergic asthma, we demonstrate that adoptive transfer of human act-A-iTr1 cells, both in preventive and therapeutic protocols, confers significant protection against cardinal asthma manifestations, including pulmonary inflammation. Overall, our findings uncover an activin-A-induced IRF4-AhR-dependent transcriptional network, which generates suppressive human Tr1 cells that may be harnessed for the control of allergic diseases.

* *Tousa S, et al.* *Proc Natl Acad Sci U S A 2017, 114:E2891.*

This project is a collaboration between the IRB and the Biomedical Research Foundation of the Academy of Athens (GR).

Experimental priming of encephalitogenic Th1/Th17 cells requires pertussis toxin-driven IL-1β production by myeloid cells

Franesca Ronchi, Camilla Basso, Silvia Preite, Luana Perlini, and Federica Sallusto

$CD4^+$ Th17 are heterogeneous in terms of cytokine production and capacity to initiate autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE). Here we demonstrate that experimental priming of encephalitogenic Th cells expressing RORγt and T-bet and producing IL-17A, IFN-γ and GM-CSF but not IL-10 (Th1/Th17), is dependent on the presence of pertussis toxin (PTX) at the time of immunization. PTX induces early production of IL-1β by $CD11b^+CCR2^+Gr1^+$ myeloid cells, which are rapidly recruited to antigen-draining lymph nodes. PTX-induced generation of Th1/Th17 cells is impaired in IL-1β- and ASC-deficient mice and in mice in which myeloid cells are depleted or fail to migrate to lymph nodes and requires expression of IL-1R1 and MyD88 on both T cells and non-T cells. Collectively, these data shed light on the enigmatic function of PTX in EAE induction and suggest that inflammatory monocytes and microbial infection can influence differentiation of pathogenic Th1/Th17 cells in autoimmune diseases through production of IL-1β.

* *Ronchi et al.* *Nat Commun 2016, 7:11541.*

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International Short Visit Fellowship to Mariana Pereira Pinho

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Publications

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Mele, F., C. Basso, C. Leoni, D. Aschenbrenner, S. Becattini, D. Latorre, A. Lanzavecchia, F. Sallusto and S. Monticelli
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T cell immunity. Functional heterogeneity of human memory CD4(+) T cell clones primed by pathogens or vaccines.
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Lectures and Seminars

Midwinter Conference "International Symposium on Immunobiology: Metabolism - Cancer - Autoimmunity - Drug Discovery"
Seefeld (AT) / 17-21.1.2015

The Rockefeller University
Seminar: Dissecting the human T cell response in immunity, autoimmunity and allergy
New York (US) / 5.2.2015

Institut Pasteur, Journée de la Recherche Translationnelle
Paris (FR) / 13.2.2015

Keystone Symposium "T cells: Regulation and effector function"
Snowbird (US) / 29.3-3.4.2015

Annual Congress American Association of Immunologists
New Orleans (US) / 8-12.5.2015

Cold Spring Harbor Laboratory Conference: Fundamental Immunology & Its Therapeutic Potential
Cold Spring Harbor (US) / 14-18.4.2015

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

Nobel Symposium on Adaptive Immunity: Defense and Attack
Stockholm (SW) / 10-13.6.2015

ETH Zurich
Seminar: Challenges and promises of human immunology
Zurich (CH) / 26.8.2015

4th European Congress of Immunology
Vienna (AT) / 6-9.9.2015

IVth Nordic Meeting on Genetics and Pathogenesis of Immunopathological Disease
Lofoten (NO) / 20-24.8.2015

Nature conference: Immune Profiling in Health and Disease
Seattle, WA (US) / 9-11.9.2015

Zing Conference: Immune System for a Therapeutic Benefit
Toledo (SP) / 30.9 – 3.10.2015

The Weizmann Institute of Science
Guest seminar: Class and specificity of the human T cell response in health and disease
Rehovet (IL) / 12.10.2015

2015 Louis-Jeantet Symposium
Geneva (CH) / 14.10.2015

3rd Symposium of the Immunological Memory and Vaccine Forum "What´s Immunological Memory?"
Berlin (DE) / 30-31.10.2015

7th EADV Dermatological Meeting in Ticino 2015: From the bench to the clinic
Bellinzona (CH) / 26.11.2015

Clinical Immunology Society, Annual meeting
Boston, MA (US) / 14-17.4.2016

CHU Purpan
Seminar: Functional T Cell Subsets in Human Health and Disease
Toulouse (FR) / 12.5.2016

HSM-2 Immunology Symposium "Immunotolerance: Moving towards Clinical application"
Zurich (CH) / 17-18.5.2016

Italian Society of Immunology, Clinical Immunology and Allergology (SIICA), Annual Congress
Abano (IT) / 25-28.5.2016

Gordon Research Conference "Chemotactic Cytokines"
Girona (SP) / 29.5-3.6.2016

Institut Curie
Seminar: The human T cell response in health and disease
Paris (FR) / 13.6.2016

Gordon Research Conference "Immunochemistry & Immunobiology"
Barga (IT) / 19-24.6.2016

La Jolla Institute for Allergy & Immunology
Seminar: Memory T cells in protective and pathogenic immune responses
La Jolla, CA (US) / 19.7.2016

International Congress of Immunology 2016
Melbourne (AU) / 21-26.8.2016

Centre d'Immunologie de Marseille Luminy (CIML) 40 Years Anniversary
Marseille (FR) / 14-17.9.2016

International Conference on Human and Translational Immunology
Rhodes (GR) / 16-21.9.2016

4th Annual Immunogenomics Conference, HudsonAlpha Institute for Biotechnology
Huntsville, AL (US) / 26-28.9.2016

Cold Spring Harbor Asia meeting "Frontiers of Immunology in Health & Diseases"
Awaji Island (JP) / 3-6.10.2016

AMED Seminar, University of Tokyo
Seminar: T cell functions in autoimmunity and immunodeficiencies
Tokyo (JP) / 6.10.2016

14th International Dendritic Cell Symposium
Shanghai (CN) / 14-18.10.2016

4th International Cytokine and Interferon Society (ICIS)
San Francisco, CA (US) / 16-19.10.2016

SIICA, SSAi, DGfI conference on Viral Immunity
Milan (IT) / 20-21.10.2016

French Society of Immunology (SFI), Annual Meeting 50th Anniversary
Pierre Grabar Lecture: Human T cell repertoires in immunity, autoimmunity and allergy
Paris (FR) / 28-30.11.2016

45th Annual Meeting of the Japanese Society for Immunology
Okinawa (JP) / 5-7.12.2016

Inselspital Bern
Seminar: Autoreactive T cells in narcoleptic patients
Bern (CH) / 11.1.2017

6th NIF Winter School on Advanced Immunology in Singapore
Singapore / 22-26.1.2017

Ragon Institute of MGH, MIT and Harvard
Seminar: Human T cell repertoires in immunity and autoimmunity
Cambridge, MA (US) / 14.2.2017

Harvard Medical School
Immunology Seminar: Human T helper cell differentiation in health and disease
Boston, MA (US) / 15.2.2017

37th European Workshop for Rheumatology Research
Athens (GR) / 2-4.3.2017

NIH Immunology Interest Group Seminar Series
Seminar: Human T cells in immunity, autoimmunity and immunodeficiencies
Bethesda, MD (US) / 3.2017

Keystone Symposium "Immune Regulation in Autoimmunity and Cancer"
Whistler (CA) / 26-30.3.2017

Henry Kunkel Society Meeting "Human Immunology and Primary Immunodeficiencies"
New York (US) / 30.3-1.4.2017

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

Orchestrated by the chemokine system, in adults 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 development and neovascularization. Within the chemokine system, CXCL12 and CXCR4 have emerged as particular couple functioning as critical homeostatic regulators of lympho-, myelo- and erythropoiesis, however the couple can also be involved in inflammatory responses. Moreover, the CXCL12/CXCR4 axis is essential for development and is involved in the growth and spreading of many tumors. Genetic deletion of either molecule leads to a comparable lethal phenotype.

Locally produced chemokines are presented on the surface of neighboring tissue to form haptotactic gradients in close vicinity of the source (~100-150µm) on which cells can migrate through the activation of G-protein coupled chemokine receptors. For the maintenance and local confinement of gradients is necessary that sinks are juxtaposed to the source of attractant. Atypical chemokine receptors (ACKRs) were recently defined as a group of receptors, which mainly act as sinks and through this activity can promote cell migration. Local scavenging of chemokines not only generates chemotactic gradients, but also prevents congestion in cell trafficking. ACKR3 binds with high affinity CXCL12 and with somewhat lower affinity CXCL11. Due to its about tenfold higher affinity for CXCL12, ACKR3 can regulate the availability of the chemokine for CXCR4. The team investigates the role of ACKR3 in the formation and maintenance of local CXCL12 gradients in lymphoid tissue for the generation of efficient humoral immune responses; the potential signaling capacity of ACKR3; and the role of the receptor in lymphoma.

Most chemokine receptors follow a common paradigm of Gi-protein coupled receptor-mediated cell activation. ACKRs share the heptahelical structure of rhodopsin-like chemokine receptors, but do not couple to G-proteins. Despite the lack of signaling through G-proteins, ACKR3 may use biased signaling though arrestin. The laboratory works on the elucidation of common and selective receptor activated pathways, investigating the molecular composition of the receptor proteomes of CXCR4 and ACKR3.

Team

Group Leader: Marcus Thelen, PhD > marcus.thelen@irb.usi.ch

Members: Sabrina Casella, PhD student - Rafet Ameti, PhD student - Diego Pizzagalli, PhD student - Viola Puddinu, PhD student - Serena Melgrati, Bachelor 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) can display a signaling bias depending on the ligand and the local environment. Such signaling bias leads to the differential activation of pathways, such as downstream of G-proteins and arrestin. Ligand-induced receptor active states represent conformations to which receptor associated proteins bind with different affinities. Thus, the interaction of GPCRs with different proteins represents most probably 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 oxidizes biotin phenol to a radical, which leads to the biotinylation of proteins in close proximity. Figure 1 shows APEX2 mediated biotinylation of proteins in transfeted HEH cells. We developed a protocol with our collaborators at the ETH Zurich (Wollscheid/ Milani) to recover with high efficiency and specificity biotinylated proteins from whole cell lysates for identification by mass spectrometry. The project is ongoing.

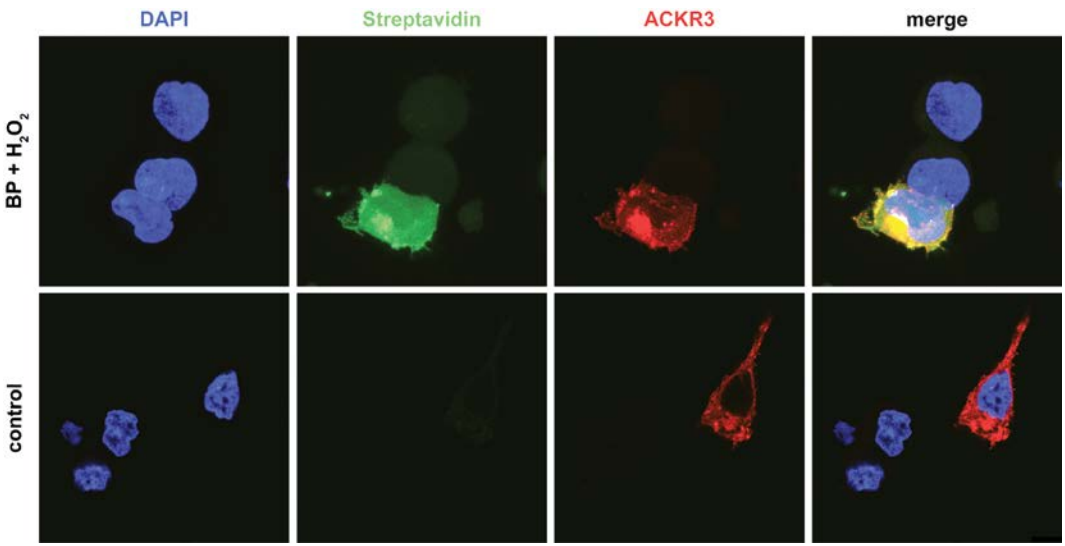

Figure 1
ACKR3-SV5-APEX2 expressed in HEK cells. In the presence of biotin phenol and H2O2 (BP + H2O2) biotinylated proteins can be visualized with fluorescent labeled streptavidin.

ACKR3 expression in B cells

Egle Radice, Rafet Ameti and Marcus Thelen

– *Functional role of ACKR3 in B cells*. Expression of ACKR3 on primary human and mouse B cells is now established. We used complementary methods including mass spectrometry to provide unequivocal evidence of the expression of ACKR3 on B cells. We showed a functional role of ACKR3 at late stages of B cell maturation, when B cells differentiate into antibody-secreting plasmablasts before homing to the bone marrow or to the mucosa and become long-lived plasma 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 line with this, we showed that plasmablast migrate more efficiently towards CXCL12 when ACKR3 is attenuated by a specific monoclonal antibody. We are currently comparing the immune responses after vaccination in wild type mice and animals with conditional deletion in different B cell compartments. Following immunization, we expect that the lack of ACKR3 in plasmablasts delays the egress of the cells from the germinal centers and attenuates the homing of long lived plasma cells in the bone marrow, hence leading to a reduced humoral protection.

– *Expression of ACKR3 in the spleen.* Early transcriptome analyses of mouse B cells indicated a marked expression of ACKR3 in splenic marginal zone B cells. Using an ACKR3$^{+/GFP}$ reporter mouse we confirmed the expression of ACKR3 in marginal zone B cells. The cell lining the white pulp face the red pulp to sample for blood born antigens. Once the cell have captured antigen, marginal zone B cells deliver antigen to the follicular dendritic cells localized in the germinal centers. The figure shows the GFP^{+} marginal zone B cells (yellow in the second row as merge of red and green) which stain also for IgM and the complement receptor CD21. Inhibition of ACKR3 was shown to cause the distortion of the microarchitecture. Current investigations aim in understanding the role of ACKR3 for the marginal zone formation and maintenance.

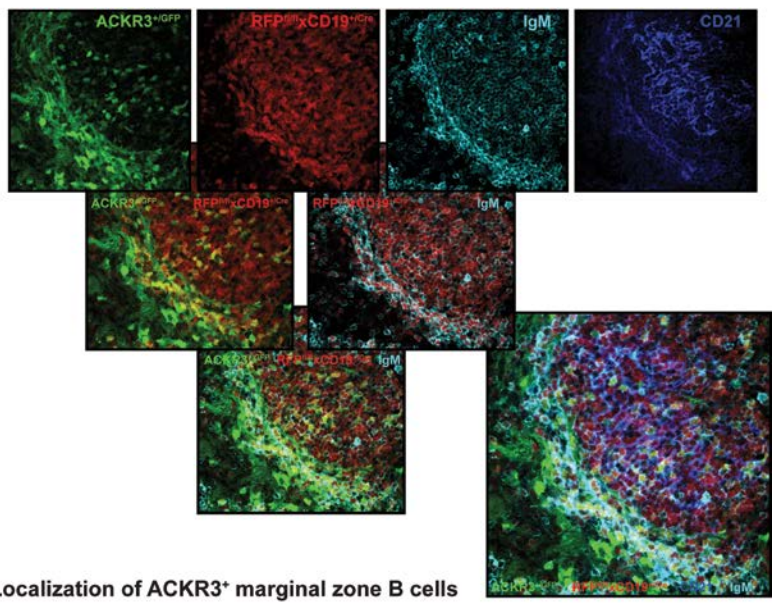

Figure 2
Frozen section of a spleen from an ACKR+/GFP x RFPfl/flCD19+/cre mouse. B cells express RFP (red) and marginal zone B cells express in addition GFP (green). The section was stained with anti IgM (magenta) and anti-CD21 blue. Image taken with Leica SP5 confocal microscope at 20x magnification and 3.8 electronic zoom.

Role of ACKR3 in cancer

Viola Puddinu, Egle Radice, Sabrina Casella, Sylvia Thelen 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. Early diagnosis of confined tumors is often associated with a good prognosis. By contrary dissemination of the primary tumor leads to a poor prognosis. Dissemination and organ infiltration of lymphomas requires migration of tumor cells from primary sites to niches where optimal growth and survival conditions are found. This migration depends on 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 cancer cells. Most studies focus on solid tumors of mesenchymal cell origin. We have shown the expression of ACKR3 on 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. B cell lymphomas originate from distinct stages of B cell differentiation and often continue to carry and exploit these characteristics as a program to survive and expand. The germinal center reaction where somatic hypermutations and class switch recombination occur is prone for the generation of neoplasms, such as CGB-Diffuse large B cell lymphoma (DLBCL). We investigate the expression of ACKR3 in respect to its effects on modulating CXCR4-dependent responses. CRISPR/Cas9 mediated elimination of ACKR3 in the human ACKR3^{+} VAL cells leads to a marked amelioration of the clinical score in a diffused xenotransplant model and a marked reduction of tissue infiltration.

In vivo studies of ACKR3 function

Rafet Ameti, Serena Melgrati, Sylvia Thelen and Marcus Thelen

Antibodies convincingly detecting G-protein coupled receptors (GPCRs) on live cells are difficult to obtain. In mice, the lack of suitable antibodies of impedes monitoring of the expression of chemokine receptors on leukocytes. We generate fluorescent-labeled chemokines to measure receptor expression and activity. We express recombinant chemokines in *E. Coli* with a poly-histidine tag at the N-terminus for purification and a consensus sequence for the phosphopantetheinyl transferase Sfp at the C-terminus for labeling with fluorescent-labeled Coenzyme A. Chemokines are purified from inclusion bodies on IMAC columns and the linear peptides folded in a redox buffer. After proteolytic removal of the N-terminus, chemokines are purified by reversed-phase HPLC. Chemokine preparations can essentially be labeled with any fluorescent dye conjugated to Coenzyme A. We use fluorescent-labeled CXCL12 to monitor the scavenging activity of ACKR3 in the presence of the CXCR4 inhibitor AMD3100.

Funding

Swiss National Science Foundation
Conventional and Atypical Chemokine Receptors: different mechanisms of function and common responses
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Swiss National Science Foundation
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310030_163336 / 1 / 2015 - 2018

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Swiss National Science Foundation
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Sinergia CRSII3_160719 responsible applicant, together with C. Halin, D. Legler and A. Rot
2015-2019

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Publications

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Mariagrazia Uguccioni

Chemokines in Immunity


PAI

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 was elected Member of the Bologna Academy of Science in 2009 for her studies on the relevance of chemokines in human pathology. In 2016, she has been nominated extraordinary Professor at the Medical faculty of Humanitas University (Italy). Mariagrazia Uguccioni's research covers various 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. Her group continue focusing on chemokine activities in human inflammatory diseases, tumours, and infections. Uguccioni's team is recently dissecting the mechanisms leading to chemokine synergism in leukocytes, and the modifications occurring in leukocytes from patients with chronic inflammatory conditions, which lead to dysfunction of the chemokine receptors.

Research Focus

Our research interest remains focused on cell trafficking in human physiology and pathology, with an emphasis on the mechanisms governing fine-tuning modulation of chemokine expression and activity, in order to identify novel therapeutic target for pharmacological intervention. Chemokines are secreted proteins, 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 chronic inflammatory diseases and on the role, chemokine heterocomplexes may have on the development of the disease. In parallel to the study of chemokine activities, we are now focusing on the modulation of the activity of chemokine receptors, that might occurs in chronic inflammation. One recent finding regards the modulation of the chemokine receptor CCR6 in HIV 1 infection. These studies might shed new light on novel pharmacological interventions aimed at favouring resolution of inflammation, or restoring chemokine receptor activities in persistent infections.

Team

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Chemokines: Structure/Function Studies

Gianluca D'Agostino, Lorenzo Raeli, Valentina Cecchinato, 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. We and other groups, to provide an explanation for the synergy between chemokines, have proposed several mechanisms: Dual receptor-mediated chemokine synergy, chemokine heterocomplexes and their interaction with Glycosaminoglycans.
After tissue damage, inflammatory cells infiltrate the tissue and release pro-inflammatory cytokines, which in turn promote the release of HMGB1, an alarmin, triggering a further release of cytokines and CXCL12 via the interaction with RAGE and TLR4. We have shown that HMGB1 enhances cell migration promoted by CXCL12, by forming a complex with the chemokine, which acts on the chemokine receptor CXCR4.

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 (IT) 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.
Moreover, we are now dissecting the mechanisms leading to enhanced migration when the complex CXCL12/HMGB1 binds to the chemokine receptor CXCR4.

* ***Cecchinato V. et al.***
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* ***Proudfoot A. and Uguccioni M.***
Frontiers in Immunology 2016, 7:article183

* ***P.J. Collins et al.***
FASEB J. 2017, published ahead of print, March 30th

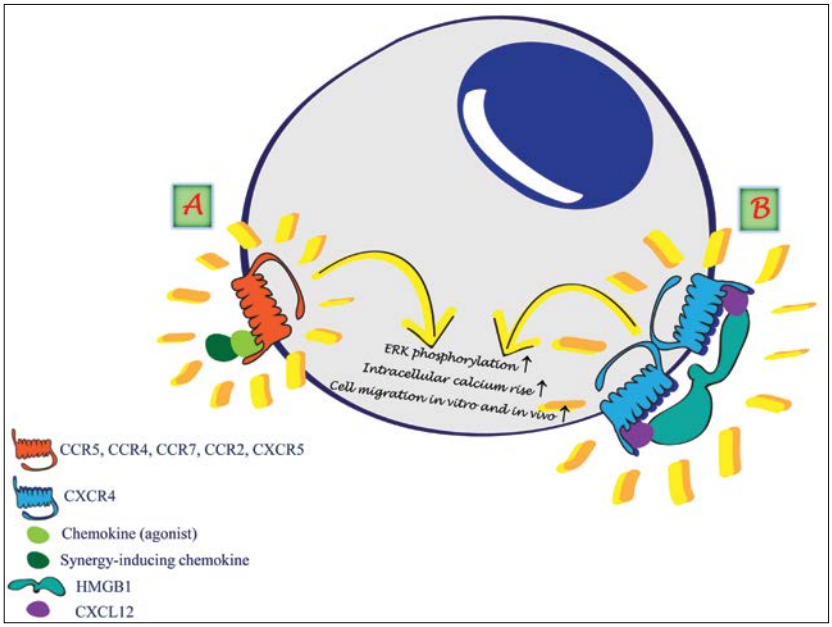

Figure 1
Model of action of HMGB1/CXCL12 synergism in leukocytes migartion. HMGB1 binds CXCL12 and the heterocomplex activates CXCR4 and induces enhanced cell migration (from: Proudfoot A. and Uguccioni M. Frontiers in Immunology 2016, 7:article183).

Responses to chemokines in HIV/SIV infection, and chronic immune activation

Valentina Cecchinato, Gianluca D'Agostino, Mara Ambrosini, Estefanía Armas González, Hasnaa Rabia Mohamed Shahin, Gabriela Danelon and Mariagrazia Uguccioni

***Ferreira T.P. et al.** **
Frontiers in Immunology 2016, 7: article 95

***Cecchinato V. et al.** **
J. Immunol. 2017, 198:184-195

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. Dr. Cecchinato has described a decrease in the frequency of Th17 cells, a subset of effector T cells involved in the immune response against extracellular bacteria, in the mucosa of SIV infected animals. Nevertheless, the migratory capacity of this T cell subpopulation has never been investigate.

Chemokines are important mediators of leukocyte trafficking and function, and deregulation of their expression might contribute in part to the pathogenesis of HIV-1/SIV infection. In the frame of a projects funded by the European Community and by the Swiss HIV Cohort Study, we have investigated the mechanisms that mediate $CCR6^+$/Th17 cells trafficking and activities at mucosal sites together with their decrease in frequency during HIV/SIV infection.

These studies are still ongoing to characterize the molecules that can be target of novel therapies not only in HIV but also in all pathological conditions characterized by persistent infections and chronic immune activation.

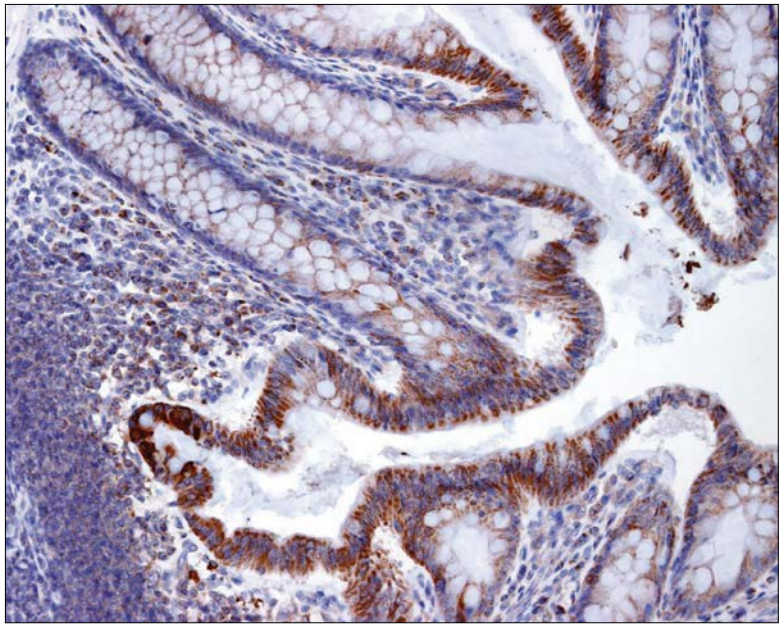

Figure 2
Expression of CCL20 in the intestinal mucosa allows Th17+CCR6+ cells to migrate into the lamina propria.

Funding

European Union
ADITEC: Advanced Immunization Technologies
FP7 –280873 / 2011-2017

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

Swiss National Science Foundation
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San Salvatore Foundation
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2013-2016

Gottfried und Julia Bangerter-Rhyner Foundation
Dampening Inflammation in Autoimmunity by Targeting Chemokine synergy-inducing molecules
2014-2016

Ceschina Foundation
The role of chemokines in tuning the inflammatory responses in Ankylosing Spondylitis
2016-2018

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Publications

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Collins, P. J., M. L. McCully, L. Martinez-Munoz, C. Santiago, J. Wheeldon, S. Caucheteux, S. Thelen, V. Cecchinato, J. M. Laufer, V. Purvanov, Y. R. Monneau, H. Lortat-Jacob, D. F. Legler, M. Uguccioni, M. Thelen, V. Piguet, M. Mellado and B. Moser
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Impairment of CCR6+ and CXCR3+ Th Cell Migration in HIV-1 Infection Is Rescued by Modulating Actin Polymerization.
Cecchinato, V., E. Bernasconi, R. F. Speck, M. Proietti, U. Sauermann, G. D'Agostino, G. Danelon, T. Rezzonico Jost, F. Grassi, L. Raeli, F. Schoni-Affolter, C. Stahl-Hennig, M. Uguccioni and H. I. V. C. S. Swiss
J Immunol. 2017; 198:184-195.

Lectures and Seminars

The first European Chemokine and Cell Migration Conference (ECMC 2015)
"Fine tuning modulation of chemokine activities by synergy-inducing molecules"
Villars sur Ollon (CH) / 06.06.2015.

12th International Conference on Innate Immunity
"Synergy in the chemokine system: a new model for tuning chemokine activities"
Crete (GR) / 22.06.2015.

IV European Congress in Immunology
"Impairment of CCR6+ and CXCR3+ T-helper cell migration in HIV-1 infection is rescued by modulating actin polymerization" (Valentina Cecchinato)
Vienna (AT) / 09.09.2015

ADITEC meeting
"Fine tuning modulation of cell migration to mucosal sites"
Bruxelles (BE) / 04.11.2015

iAR meeting
"HMGB1 controlling CXCL12 activities in Rheumatoid Arthritis"
Lausanne (CH) / 10.12.2015

AIBT 2016
"Chemokines in Immunity"
Pesaro (IT) / 10.06.2016

International HMGB1 Workshop
"HMGB1 and the chemokine system: a new model for tuning chemokine activities"
Milan (IT) / 13.10.2016.

Humanitas University
"Modulation of Leukocyte Migration in Health and Disease"
Milan (IT) / 13.01.2017

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) and obtained a PhD degree at the prestigious MRC-Laboratory of Molecular Biology (University of Cambridge, UK) using molecular and structural biology to study RNA-protein interactions. He contributed to show the key role played by RNA in regulation of gene expression and how RNA itself can be a valid therapeutic target against dementia. His numerous high caliber publications, culminated in the determination of the largest NMR structure available at the time, allowed him to move to Stanford with a "long term EMBO fellowship", reserved to the best young molecular biologists in Europe. In California Luca Varani completed the first magnetic resonance study on TCR/pMHC, key proteins of the immune system. Since October 2007 he leads the Structural Biology group of the Institute for Research in Biomedicine (Bellinzona, CH). The main activity involves the characterization of interactions between pathogens and antibodies, molecules of the immune system capable of curing and protecting from illness. The group tries to understand the molecular properties that allow a given antibody to eliminate a pathogen. Studies involve mainly rare and neglected diseases such as Dengue or Zika virus, Prion or rare form of Leukemias. The NMR approach developed at Stanford was pushed forward at the IRB, where computational techniques allow discovering which part of the pathogen is recognized by antibodies. Experimentally guided and validated computational simulations yield the atomic three-dimensional structure of antibody/pathogen complexes. The approach allowed to rationally modify an existing antibody, increasing its ability to neutralize Dengue virus by 50 fold utilizing, for the first time, only computational tools. They also performed one of the rare NMR studies showing how antibody binding alter the local flexibility of the antigen. The group uses a highly multidisciplinary approach, varying from structure determination to cellular experiments, from computational biology to protein and antibody production and engineering, from synthesis of nanoparticles to confocal microscopy.

Research Focus

Our group uses computational, biochemical and biophysical tools to determine the three-dimensional atomic structure of proteins and characterize their interactions with other molecules, with particular attention to antibody-antigen interactions in infectious diseases. The final goal is to understand the molecular properties that make a given antibody effective against a pathogen, and eventually to exploit this knowledge to design new drugs against diseases such as Dengue and Zika, Prion or some rare form of Leukemia. Dengue and Zika are tropical viruses in rapid expansion whereas Prion, famous in the 90s due to the Mad Cow scare, causes a neurodegenerative disease with no cure and still largely unknown. Understanding which part of the pathogen is recognized by the most efficient antibodies allows discovering and blocking of the key parts of the pathogen itself. Our group has a highly multidisciplinary approach that merges biochemical data, experimental structural information and computational simulations. Computational Structural Biology, in particular, is a rapidly developing and increasingly important field. At this time, however, computational predictions are not always accurate; it is therefore crucial to guide and validate them with experimental data. The synergy between computational simulations and classic biophysics, molecular and cellular biology combines the best of both approaches: the low cost and high speed of computers with the rigorous and reliable experimental validation. It is common opinion among scientists that future biomedical sciences will require a combination of computational and experimental techniques.

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Characterization of antibody-pathogen interactions

Luca Simonelli, Mattia Pedotti, Marco Bardelli, Daniela Iannotta 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, and Humabs); characterize their immunological and biophysical properties; determine their epitope through NMR epitope mapping or mutagenesis and use the experimental results to drive and validate computational docking simulations of their complex with the desired antigen. Finally, 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 and Zika Virus

Dengue (DENV) and Zika (ZIKV) are flaviviruses with similar biological and structural features.

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. ZIKV has recently seen epidemic outburst and has been linked to fetal abnormalities. Both viruses are considered major health threats and no cure or vaccine is available. Although being mainly restricted to the tropical region, both their epidemic activity and 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.

We are studying how potent, neutralizing human monoclonal antibodies interact with these viruses, searching for correlations between immunological and structural trends and exploiting them to further our understanding of antibody-antigen interaction.

A combination of NMR epitope mapping, site directed mutagenesis and computational simulations allow us to determine the three dimensional structure of antibody/antigen complexes. Confocal microscopy, cellular infectivity and biological assays teach us which part of the viral infection cycle is hindered by antibody binding.

In the recent past we have been able to use the above information to introduce rational mutations in an anti-Dengue antibody and i) alter its specificity ii) improve its neutralization potency by up to 50 times. This was achieved, for the first time, without the aid of crystallographic structures, proving that experimentally validated computational docking is an accurate, rapid and powerful tool for the characterization and rational engineering of antibodies.

Structural Characterization of potent neutralizers of Dengue and Zika Virus

DV87 is possibly the most potent Dengue antibody described so far in the literature. It binds the so called DIII of the surface protein of Dengue virus and potently neutralizes it (IC50 4 ng/ml). Most 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 discovered that this is not the case; on the opposite, its novel epitope is totally inaccessible on the viral structure. Antibody binding requires a local conformational change that, in turn, disrupts the global viral structure and prevents it from infecting cells.

Similarly, antibody ZKA190 is an extremely potent human antibody binding to DIII and protecting from ZIKV infection. Through NMR epitope mapping, computational simulations and cryo electron microscopy we were able to show that ZKA190 binds to a novel epitope on DIII, altering the quaternary viral structure through allosteric effects and preventing the E protein conformational change required for viral fusion to the target cell membrane. Distortion of the viral structure by antibody binding allows 180 copies of ZKA190 to simultaneously bind to the virus, a rare property likely to contribute to the antibody efficacy.

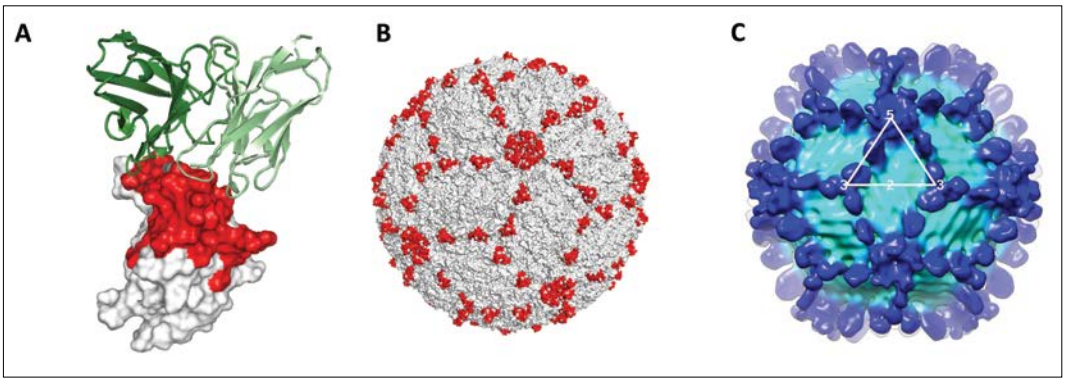

Figure 1

The human antibody ZKA190 binds to the surface protein of Zika and potently neutralizes the virus. A) Surface representation of DIII of the ZIKV Envelope protein; the antibody is in green and the NMR derived epitope in red. B) The epitope is colored red on the viral surface. C) Cryo-EM structure of ZKA190 (blue) bound to Zika virus (cyan); all 180 available binding sites are occupied.

Antibody Binding modulates conformational exchange in Dengue Virus E protein

Structural flexibility is an important element in protein-protein interactions, and yet studying its role in antibody-antigen recognition has been largely neglected, mainly because the subject is difficult to approach by x-ray crystallography, which is traditionally used to observe antibody complexes.

By contrast, solution NMR spectroscopy provides direct, residue-level measurements of flexibility but it is difficult to apply to antibody/antigen complexes due to technical difficulties.

In a rare example of NMR study on antibody complexes, we provided a dynamic description of DIII from two different Dengue serotypes either free or in complex with a neutralizing antibody. We observed conformational exchange in the isolated DIII, in regions important for the viral quaternary packing. This conformational diversity is likely to facilitate the partial detachment of DIII from the other E protein domains, which is required to achieve fusion to the host cellular membranes and to expose the epitopes of many anti-DIII antibodies. Antibody binding to DIII attenuated the conformational exchange in the epitope region, in line with a model of conformational selection by the antibody. Surprisingly, however, antibody binding generated exchange in other parts of DIII through allosteric effects. This may be a mechanism to compensate the entropic cost of epitope rigidification by allowing increasing flexibility in other antigen regions. The allosteric effects on DIII facilitate the disruption of intermolecular contacts on the viral surface, altering the viral quaternary structure.

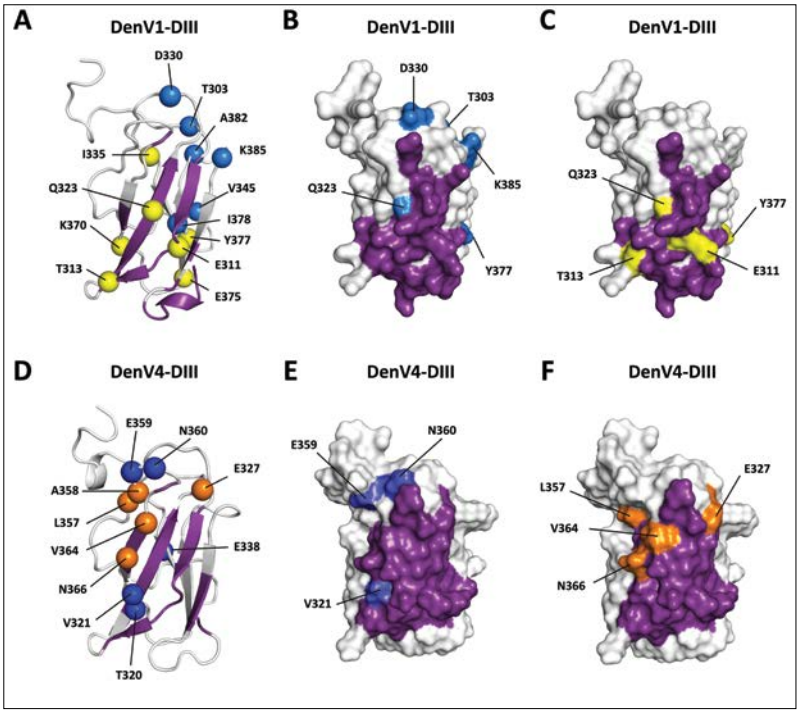

Figure 2

Antibody binding to Dengue virus quenches conformational exchange of epitope residues in a process reminiscent of conformational selection, but causes allosteric conformational exchange outside the epitope.

Cartoon and surface representation of DIII of the surface Envelope protein of Dengue Virus are shown (top, DENV1; bottom, DENV4). The NMR derived epitope of a human neutralizing antibody is in purple. Residues showing conformational exchange in the free antigen, according to NMR dynamics measurement, are in yellow and orange. Residues with conformational exchange in the antibody/antigen complex are in blue.

Antibodies as a tool to investigate prion protein toxicity

Marco Bardelli, Luca Simonelli, Mattia Pedotti 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 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 have also engineered a bispecific antibody binding simultaneously to the globular domain and flexible tail of prion protein. This molecule is one of the most effective antibodies protecting against prion neurotoxicity, being able to prevent scrapie mediated prion toxicity even when administered 21 days post infection.

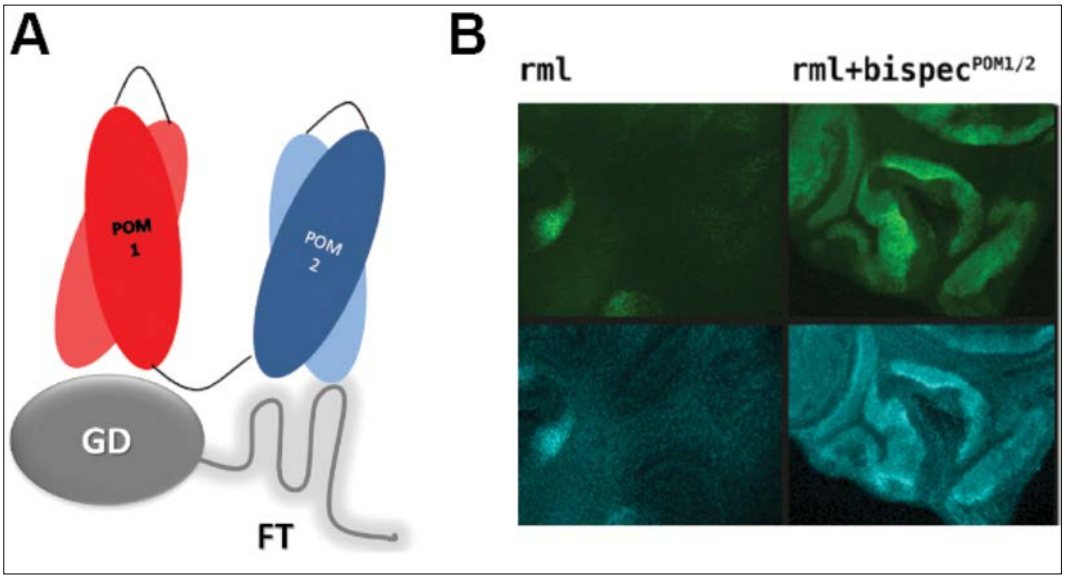

Figure 3
A bispecific antibody potently protects from prion induced neurotoxicity.
A) schematic representation of the bispecific simultaneously targeting the globular domain (GD) and flexible tail (FT) of prion protein. B) Cellular assays to evaluate neuronal health. Addition of toxic, RML prion (left) causes neuronal death, as indicated by the low level of NeuN staining. Right: toxicity is not detected in the presence of the protective bispecific antibody.

Targeting Acute Myeloid Leukemia with antibodies

Marco Bardelli, Luca Simonelli, Mattia Pedotti 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. CD123 and Tim3 are viable targets in acute myeloid leukemia (AML), a cancer with a high recurrence of relapses due to the presence of Leukemic Stem Cells that are not affected by normal chemotherapic drugs.

We are investigating two approaches, CAR immunotherapy and nano-vector based therapy, for selectively targeting AML stem cells with a bio-recognition element (antibody) capable of discriminating them from normal, healthy cells.

In CAR immunotherapy the antibody is linked to an engineered T-cell chimeric antigen conferring to such cells the ability to recognize, and subsequently kill, tumor cells. We are studying how the CAR efficacy and safety is affected by the affinity of the targeting antibody for the target antigen. Lower affinity, in fact, might avoid recognition and killing of healthy cells with low expression of the target antigen. Rational engineering of antibody mutants with different binding affinity allows us to observe the effect of antibody binding independently of other factors such as epitope recognition, nature of the linkage between antibody and modified T-cell and other factors that remain identical in all our constructs.

In a second approach, we are building an antibody-nanoparticle-drug construct to achieve targeted delivery of chemotherapy agents to leukemic cells. AML treatment requires the usage of high doses of cytarabine, a chemotherapy drug. Frail, young and elderly patients cannot tolerate such dosage due to side effects, with very negative prognosis. Decreasing the toxicity of cytarabine, even marginally, would have a clear therapeutic advantage.

By linking the drug to an antibody that recognizes surface antigens overexpressed on AML cells, it is possible to preferentially deliver the drug to cancer cells, avoiding its interaction with healthy cells and consequently limiting its toxicity. In practice, the antibody is attached to a nanoparticle that contains the drug and releases it only within the target cells. The nanoparticle increases cellular drug uptake, *in vivo* and on shelf stability.

Preliminary results in in vitro models indicate that our antibody-nanoparticle-drug conjugate can kill ~30% more target (cancer) cells then the drug used for AML treatment. At the same time, our conjugate kills ~30% less healthy cells than the currently available drug.

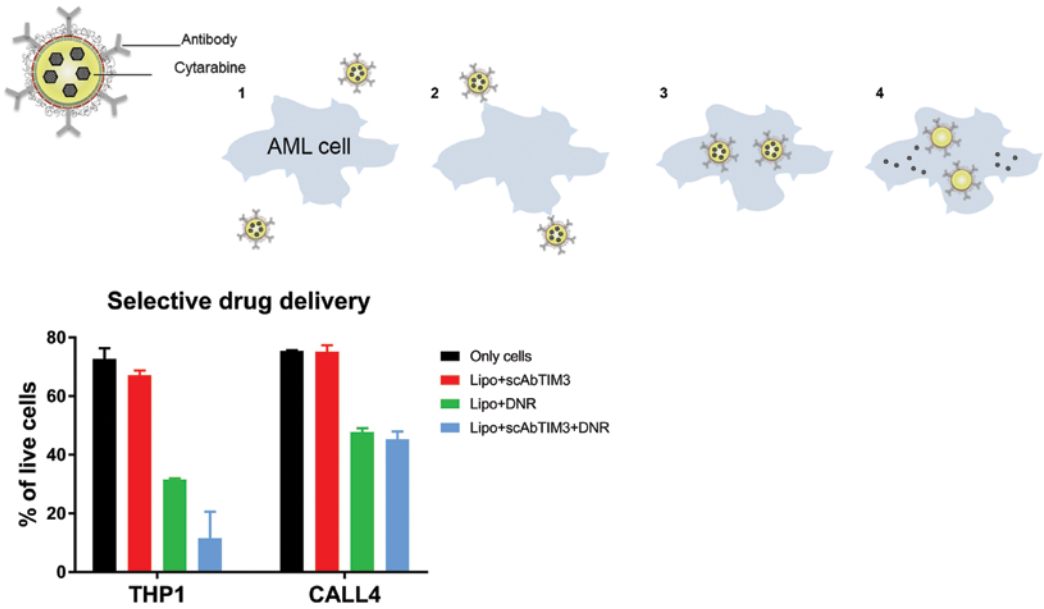

Figure 4

Antibodies that recognize cancer protein markers expressed on the surface of leukemia (AML) cells allow the selective delivery of chemotherapy drugs to cancer cells, limiting the toxicity related to undesired targeting of healthy cells.
Top: Schematic representation of our antibody-nanoparticle-drug construct. The chemotherapy drug (cytarabine) is encapsulated in a nanoparticle to which a targeting antibody is attached. The antibody targets leukemik cells (steps 1 and 2) and the nanoconjugate is internalized (3). At this point the nanoconjugate opens up and releases cytarabine (4).
Bottom: cellular toxicity assays show that our nanoconjugates kills more target AML cells (THP1, green bar) than the drug alone (THP1, cyan bar). The difference is not detected on non-target cells (CALL4, green and cyan bar).

Other collaborative projects

In collaboration with M. Molinari (IRB, CH), we identified the role of protein Sec62 during recovery from ER stress to selectively deliver ER components to the autolysosomal system for clearance. We used a combination of NMR mapping, computational simulations and SPR assays to show that Sec62 directly interacts with LC3 protein through a conserved region required for its function in ER stress recovery.

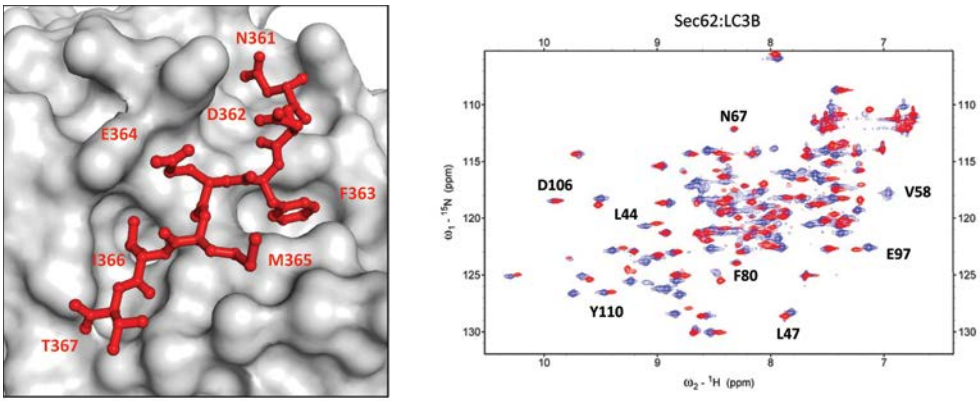

Figure 5
Structure of the Sec62 LIR peptide (red) bound to LC3 protein (grey surface) obtained by computational simulations guided and validate by experimental NMR mapping. The NMR spectra of LC3 free (blue) and peptide bound (red) are overlaid on the right. Some of the LC3 residues affected by peptide binding are labelled.

Building upon our experience in the characterization of antibody-antigen complexes, in collaboration with A. Lanzavecchia (IRB, CH) we used a combination of rational mutagenesis and computational simulations to obtain a three-dimensional model of the complex between a human antibody and the surface protein of MERS coronavirus. This is a highly lethal pulmonary infection caused by a recently identified virus. The antibody binds to a novel site on the viral spike protein and potently neutralizes infection of multiple MERS-CoV isolates by interfering with the binding to its cellular receptor.

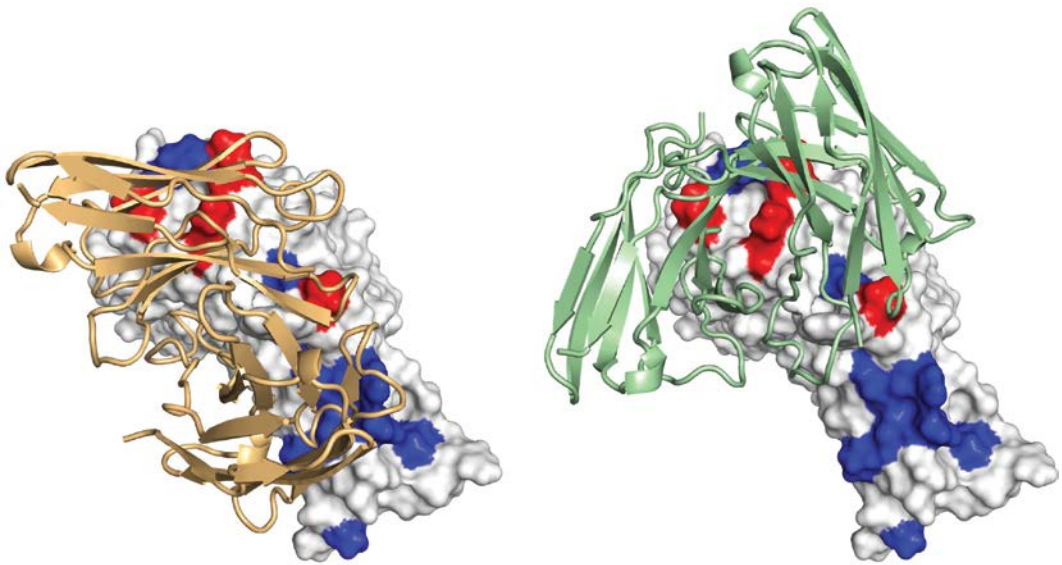

Figure 6
Computational structure of a human MERS neutralizing antibody (orange and green) bound to the surface of MERS coronavirus. Two equally valid solutions according to the computational results are shown. Site directed mutagenesis indicates that the right structure is in better agreement with the experimental information. Antibody binding, in fact, is inhibited when antigen residues shown in red, but not in blue, are mutated.

Funding

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

Swiss National Science Foundation
Characterization of antibody-antigen interactions in human pathogens
310030-166445 (2016-2019)

Swiss Cancer League
Targeted delivery of chemotherapy agents to Acute Myeloid Leukemia cells by antibody-nanoparticle conjugates
KSF-3728-08-2015 (2016-2018)

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

KTI project Nr. 15524.1 VOUCH.LS
Mass Virus Typer: New Technology for Direct Virus Characterization (2013-2015)

Swiss National Science Foundation
Acquisition of a 600MHz solution NMR spectrometer
316030-157699
2015

Collaborations

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Tettamanti Research Centre, Monza Hospital (IT)

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

CSCS, Swiss Supercomputer Center
Manno (CH)

Stephane Follonier
CSEM Landquart (CH)

Humabs (CH)

Antonio Lanzavecchia
Institute for Research in Biomedicine (CH)

Maurizio Molinari
Institute for Research in Biomedicine (CH)

Lok Shee Mei
Duke-NUS Medical School (SG)

Vadim Sumbayev
University of Kent (UK)

Ana Paula Valente
University of Rio de Janeiro (BR)

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J Mol Recognit. 2015; 28:393-400.

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Wyszynski, R. W., B. F. Gibbs, L. Varani, D. Iannotta and V. V. Sumbayev
Cell Mol Immunol. 2016; 13:47-56.

Lectures and Seminars

Congress at University of Padova
"Characterization and Engineering of human anti Dengue antibodies"
Padova (IT) / April 2016

AUREMN, 15th South American NMR meeting
"Characterization and Engineering of human anti Dengue antibodies"
Angra dos Reis (BR) / June 2015

Presentations to general public:

Lecture to high school students
Bellinzona (CH) / March 2016

Lecture to Lions Club
Bellinzona (CH) / June 2015

Lecture to high school students
Bellinzona (CH) / March 2015

CORE FACILITIES

SECTION 2

INDEX SECTION 2 – CORE FACILITIES

The imaging facility

The imaging facility (Flow cytometry and microscopy) is headed by David Jarrossay who obtained a PhD degree at the University of Fribourg (CH). It is central to most of the research projects.

The IRB has a state-of-the-art flow cytometry facility that is run by David Jarrossay who takes care of the cell sorting, maintenance of the equipment, instruction and advice to the new operators in addition to performing his own research.

*Rieckmann, J.C. et al. **
Nat. Immunol. 2017, 5:683-693

Rocco D'Antuono, recruited in 2014, is a microscopist and image analyst and runs the microscopy facility. He has experience in confocal microscopy and high content cellular analysis.

*Tan, J. et al. **
Nature 2016, 7:105-9

Flow cytometry lab

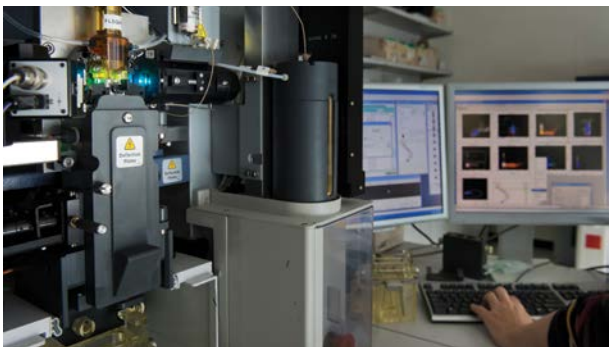
The Flow Cytometry lab provides investigators with equipment and support for cell sorting (separation), acquisition, and analysis of flow cytometric data with a variety of state-of-art multicolor flow cytometry instruments.

– **Cell sorting**
  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 iQue (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 lab 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. Three stations for data analysis (FlowJo software) are also available.



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 (Figure 3), deconvolution and 3D reconstruction; thanks to a wide range of software such as ImageJ, CellProfiler, RStudio, MetaMorph (Molecular Devices), Matlab (MathWorks), Imaris (Bitplane).

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.

– **Multiphoton 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).

* ***Chantziandreou N. et al.***
Cell Rep. 2017, 18:2427-2440

* ***Fumagalli F. et al.***
Nat. Cell Biol. 2016, 18:1173-1184

* ***Leoni C. et al.***
Eur. J. Haematol. 2016, 95:566-75

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.

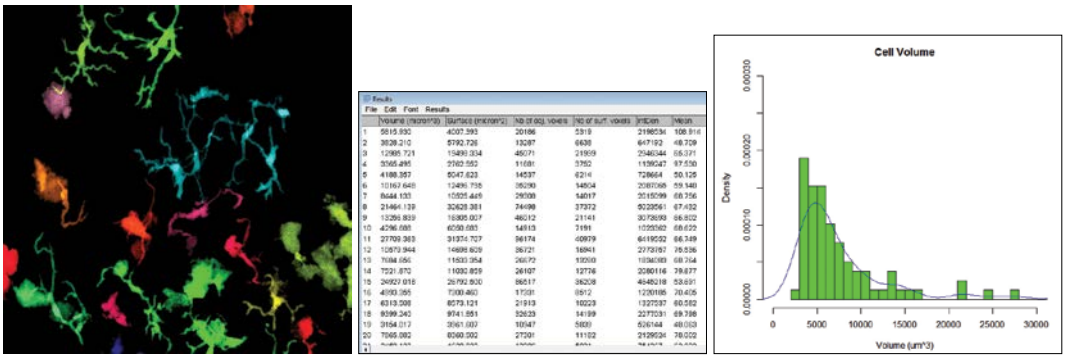


Figure 3
Example of image analysis: color-coded representation of segmented cells and statistics.

Gene Expression and Protein Purification Platform.

The research aim of IRB Gene Expression and Protein Purification Platform (GEPP) is molecular recognition, which plays a crucial role in the fields of infectious diseases and immunology. Molecular recognition intervenes in antigen-antibody or antigen-TCR interaction, synergistic effects in biological systems and host pathogen interactions.

We recently investigated molecular mechanisms leading to human cytomegalovirus (HCMV) entry[1] and spreading[2]. We also use HCMV as a model to understand receptor mediated viral infection and develop novel strategies for pathogen receptor identification. In addition, we are currently focusing on nanoparticles based vaccine development, addressing the unmet medical need for a human respiratory syncytial virus (RSV) vaccine. For our research projects, we use a variety of approaches from biochemistry/biophysics to molecular and cellular biology.

We provide training, help and expertize to IRB scientists for proteins/antibodies analysis, expression, purification[3] and biophysical analysis[4], including: protein/protein and protein/molecule interactions by Isothermal Titration Calorimetry (ITC) and/or Surface Plasmon Resonance (SPR), Circular Dichroism (CD), Dynamic Light Scattering (DLS), and High-Performance Liquid Chromatography (HPLC). We also have experience with the Hybridoma technology to generate murine monoclonal antibodies against proteins of interest.

The team currently collaborates with different groups inside and outside of the IRB (academic centers and industries) on multiple projects. We are opened to novel collaborations on host pathogen interactions, vaccine discovery or additional projects with biomedical interest.

* *Kabanova, A. et al.* *Nat Microbiol. 2016, 1:16082*

* *Gerna, G. et al.* *J. Virol. 2016, 90:6216-6223*

* *Corti, D. et al.* *Science. 2016, 351:1339-1342*

* *Brambilla, L. et al.* ***Mol. Oncol. 9:1194-1206***

PhD PROGRAMME
SECTION 3

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Giorgia Brambilla Pisoni

In vivo characterization of TMX1: TMX1 preferentially acts upon transmembrane polypeptides

Supervisor: Maurizio Molinari // Co-referees: Markus Aebi, Paola Picotti, Roberto Sitia
Swiss Federal Institute of Technology, Zurich, Switzerland

The endoplasmic reticulum (ER) is a complex organelle divided into different sub-compartments that are required for the execution of diverse functions, such as protein and lipid synthesis, Ca++ storage and drug detoxification. The rough ER (RER) is the subdomain designated for the production of secretory and membrane proteins in eukaryotic cells, which constitute about one third of the total proteome of the cell.

***Brambilla Pisoni, G., et al.** **
Mol. Biol. Cell. 2015, 26: 3390-3400.

Protein translocation into the ER represents the first stage of protein secretion. Upon ER import, the vast majority of nascent polypeptides are modified by the oligosaccharyltransferase complex (OST), which catalyzes the addition of a pre-formed 14-subunits oligosaccharide (3 glucoses, 9 mannoses and 2 N-acetylglucosamines) specifically on the -N-X-S/T/C- consensus sequences displayed by the polypeptide chains. This process is called N-glycosylation and is responsible for both the increase in solubility of the polypeptides and the creation of binding motifs for ER resident lectins that drive glycopolypeptide folding.

***Brambilla Pisoni, G., et al.** **
Traffic. 2016, 17: 341-350.

Once the nascent polypeptide has been N-glycosylated, glucoses and several mannose residues are sequentially removed by ER-resident glycosyl hydrolases in a thinly-regulated process known as glycan trimming which dictates the glycopolypeptide fate until its complete maturation or selection for degradation.

***Bergmann, T. J., et al.** **
AIMS Biophysics. 2016, 3: 456-478.

Maturing mono-glucosylated polypeptides enter the folding program principally managed by the ER lectins calnexin (CNX) and calreticulin (CRT). CNX and CRT in turn recruit specific ER resident enzymes which assist folding rate-limiting steps. These are enzymes that catalyze the formation of native inter- and intra-disulfide bonds within the polypeptide chains, called protein disulfide isomerases (PDI), and the isomerization of peptidyl-prolyl bonds of maturing polypeptides, called peptidyl-prolyl isomerases (PPI).

The lumen of the mammalian ER contains more than 20 members of the PDI superfamily. The reasons for the high redundancy of PDI members and the substrate features required for preferential engagement of one, or the other, are poorly understood.

After the release from the CNX-CRT folding cage, a sophisticated quality control machinery guarantees that only correctly folded polypeptides are able to leave the ER via COPII-mediated vesicles destined to the Golgi compartment. Since polypeptide folding is obviously an error prone process, misfolded intermediate products can form and become extremely dangerous for the cell.

Specific sensors within the ER lumen are able to judge whether misfolded proteins can re-enter the folding program for new folding attempts or must be labelled as terminally misfolded and addressed for ER-associated degradation (ERAD), a complex series of events that comprehend polypeptide recognition, linearization for retro-translocation, poly-ubiquitilation and finally degradation by cytosolic proteasomes. Also in this context, components of the PDI superfamily have been indicated as key players during the preparation for degradation of misfolded polypeptides.

To shed light into the PDI cellular roles and specificities, we focused our attention on the PDI subgroup called thioredoxin-related transmembrane proteins (TMX). We investigated how TMX1, one of the five transmembrane members of the TMX subfamily, intervenes during the folding program of model folding-competent substrates.

We found that TMX1 forms functional complexes with CNX and preferentially intervenes during maturation of cysteine-containing membrane-associated proteins, while ignoring the same cysteine-containing ectodomains if not anchored at the ER membrane. As such, these data demonstrate that TMX1 is the first

example of a topology-specific client protein redox catalyst in living cells.
Given that a role for TMX1 during translocation of catalytic toxin subunits across the ER membrane has been proposed and that this transport derives from the ERAD machinery hijacking by exogenous pathogens, in a second study, we investigated whether TMX1 may also facilitate dislocation of misfolded polypeptides across the ER membrane for ERAD degradation.
Our results indicate that TMX1 binds to the model folding-defective membrane-bound BACE457 via mixed disulfides, decreasing its degradation's rate. As in the case of folding, TMX1's role in ERAD seems to be preferentially addressed toward membrane-bound polypeptides.

Caterina Elisa Faliti

Mechanisms of purinergic regulation during humoral immune response in physiology and pathology

Supervisor: Fabio Grassi // Co-referee: Christoph Müller, Kenneth McCullough
University of Bern, Switzerland

Extracellular ATP acts as a signaling molecule by stimulating purinergic receptors in the plasma membranes of eukaryotic cells termed P2. P2X receptors are nonselective cation channels, whereas P2Y are G protein-coupled receptors. On the other hand, extracellular levels of ATP are controlled by the expression of ectonucleotidases. The rate-limiting step of the ectonucleotidase cascade for adenosine generation is represented by CD39 (E-NTPDase-1) that hydrolyzes ATP/UTP and ADP/UDP to the respective nucleoside (e.g. AMP). In turn, extracellular nucleoside monophosphates are degraded to adenosine by the plasma membrane ecto-5'-nucleotidase CD73. In the first part of my PhD, I addressed the role of CD39 and CD73 in mouse B cells in promoting Ig class-switch recombination via generation of adenosine. We have shown that murine B cells release ATP that accumulates in late endosomal/lysosomal vesicular compartments defined by the presence of the vesicular SNARE protein TI-VAMP. B cells with Vamp7 (encoding for TI-VAMP) or Nt5e (encoding for CD73) deletion are defective in generating extracellular adenosine and switching Ig class isotype. These results were correlated with selective deficiency of CD73 expression in patients with common variable immunodeficiency (CVID) that are characterized by impaired class-switched antibody responses, suggesting that CD73-dependent adenosine generation contributes to the pathogenesis of the disease.
The second part of my PhD was dedicated to understanding the role of the ATP-gated ionotropic P2X7 receptor in regulating the B cell helper function of T follicular helper (Tfh) cells. Tfh cells induce germinal center (GC) reaction and promote Ig class switch recombination and production of high affinity antibodies. Aberrant Tfh cells expansion result in autoantibodies generation and autoimmune manifestations. It was previously shown in my host lab that P2X7 is selectively and robustly upregulated in Tfh cells from chronically stimulated secondary lymphoid organs, such as Peyer's patches and mesenteric lymph nodes, where it controls expansion of gut commensals specific Tfh cells. We addressed the possible role of P2X7 in limiting Tfh cells expansion in two immunopathological conditions. Intra-peritoneal injection of pristane induces chronic inflammation that causes a lupus-like syndrome characterized by the development of autoantibodies, polyclonal hypergammaglobulinemia and glomerulonephritis. We have shown that lack of P2X7 worsened the outcome of this syndrome, enhanced generation of autoantibodies and promoted IFN-γ secretion. These effects were dependent on lack of P2X7 activity in Tfh cells. Conversely, we did not detect alterations in the response of *P2rx7*$^{-/-}$ Tfh cells (with deletion of *P2rx7* gene encoding for P2X7) stimulated with ovalbumin as a "conventional" antigen in an immunization protocol. Circulating Tfh cells from lupus patients were

* ***Perruzza, L., et al.*** *Cell Rep. 2017, 18:2566-2575.*

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

hyporesponsive to P2X7 stimulation and resistant to P2X7 mediated inhibition of cytokine driven proliferation. These observations suggest that P2X7 constitutes an important regulator of Tfh cells function to limit possible immunopathological damage by exaggerated B helper activity.

Alexander Frühwirth

The human neutralizing antibody response against A/H1N1/Ca/2009 Influenza virus

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

The *Influenza* A virus is classified based on 11 neuraminidase (NA) and 18 hemagglutinin (HA) glycoproteins that are subclassified into two major groups called group 1, which includes H1, H2, and H5, and group 2, which includes H3 and H7. Current seasonal *Influenza* A infections are caused by two specific strains, H1N1 (derived from the 1918 and 2009 pandemics) and H3N2 (derived from the 1968 pandemic), and are the focus of current vaccination strategies due to their prevalence in the human population. The continuous drift of H3N2 has forced its vaccine formulations to be changed constantly, whereas the recent pandemic H1N1 virus (A/California/7/2009) has remained relatively stable since 2010 and has not been changed in the annual vaccines since then.

*Lanzavecchia, A., et al. **
Curr. Opin. Immunol. 2016, 41: 62-67.

The HA molecule is the primary target of the neutralizing antibody response. The HA head is the most variable region and comprises five antigenic sites which have been mapped originally on PR8 using viral escape mutants (Sa, Sb, Ca1, Ca2 and Cb). Anti-HA head monoclonal antibodies are strain-specific and are known to be highly neutralizing by steric hindrance while anti-HA stem antibodies are heterosubtypic and weakly neutralizing, but showed protecting activity *in-vivo* in a Fc-depending manner, underlining the importance of additional effector mechanisms to be considered in the assessment of immune responses.

Human monoclonal antibodies (mAb) against the stem have been extensively characterized and shown to have a convergent (public) gene usage of VH1-69, to mature to full efficiency with only a few mutations and target conserved epitopes of HA, allowing them to bind to *Influenza* group 1 and 2 viruses. In contrast it is yet unclear how anti-head antibodies mature and which V-gene families are publicly or non-convergently (privately) used against the different antigenic regions. The few in-depth human studies that have been done suggest multiple possible public VH gene usages, such as VH4-59 for antibodies against the receptor binding site or VH3-7 and VH3-15 in a focused antibody response against the border of the Sa and Ca1 region (K166, using H3 numbering). Overall, this underlines the need for further studies in humans to understand the development of the *Influenza* antibody response across the whole HA molecule in terms of public or private V-gene usage, maturation pathways and effector functions.

In this study, we analyzed the anti-HA head response of two vaccinated individuals, one of whom has been previously characterized in depth in terms of the anti-HA stem response. We isolated and characterized a panel of 95 monoclonal antibodies specific to H1N1/California/07/2009 (Ca09) in terms of their epitopes, V-gene usage, developmental processes and effector functions across the whole HA molecule.

We established, with the help of a panel of antibodies mapped via viral escape mutants, a blocking-of-binding (BoB) assay to deconstruct, quantitatively, the sera antibody response according to the targeted antigenic sites on the HA over multiple years as well as in multiple donors. We report a potential public VH2-70 lineage of anti-HA head antibodies in two anti-Sa/Sb-dominated responses and describe their development by genealogy tree reconstructions in perspective of other anti HA-head antibodies. In addition, we identified a new antigenic site of the HA stem named the membrane-proximal external region (MPER) and a novel,

functionally distinct, class of antibodies associated with it which can be found in multiple donors.
Thus, the results of this study provide novel insights into the development of anti-HA head antibodies and contribute valuable information for the construction of a universally effective anti-HA immune response in the future by adding a new and distinct class of anti-HA stem antibodies to the picture.

Fiorenza Fumagalli

Unraveling the molecular pathways controlling recovery from a transient ER stress in mammalian cells

Supervisor: Maurizio Molinari // Co-referees: Mario Tschan, André Schneider
University of Bern, Switzerland

The Endoplasmic Reticulum (ER) is a specialized organelle in eukaryotic cells where about one third of the proteome is synthesized. In this organelle molecular chaperones and folding enzymes assist the proper folding of proteins of the secretory pathway that are then delivered to their site of activity. A finely tuned mechanism exists in the ER to assure the retention of immature proteins and the disposal of misfolded proteins, a process known as ER-Associated Degradation (ERAD).
It is very important to keep the ER homeostasis under control, as disturbances of this delicate equilibrium can elicit ER stress and activation of the unfolded protein response (UPR). Such response ensures cell's survival if the stress can be coped with; otherwise UPR leads to apoptosis and elimination of the cell.
Recovery programs after resolution of a transient ER stress are activated to remove excess/damaged ER produced during the stress phase in order for the cells to return to a physiologic, pre-stress of ER volume and intra-organellar content situation.
While mechanisms acting during a stress in the ER have been extensively studied and characterized, little is known about what happens on resolution of an ER stress. Thus, in order to characterize in more detail the recovery from a transient ER stress, in the first part of my thesis I set up a protocol for reversible induction of UPR in mammalian cells. Cells were exposed for 12 h to ER stressors to elicit UPR. Following wash out of the drug, we could observe a fast return of ER stress-elicited transcripts to pre-stress levels. The return of stress-induced proteins was much slower and delayed on deletion of core autophagy genes and inhibition of lysosomal degradation.
By means of various techniques such as bioinformatic analysis, Surface plasmon resonance (SPR), Nuclear magnetic resonance (NMR) spectroscopy, peptide arrays, computational modeling and docking, confocal, correlative light electron and immune-electron microscopy, Clustered regularly interspaced short palindromic repeats/CRISPR associated proteins (CRISPR/Cas9) and mass spectrometry, the translocon protein SEC62 was identified as an ER-phagy receptor possessing a LC3-interacting region (LIR).
We could describe SEC62 as a new ER autophagy receptor that plays its role during recovery from a transient ER stress delivering to autolysosomes select portions of ER to be cleared. We named this process recovER-phagy. These findings identify SEC62 as a determining factor involved in ER homeostasis in mammalian cells.
In the second part of my thesis, I analyzed into more detail the autophagy requirements for recovER-phagy. The different steps of the autophagy pathway were analyzed: select members of the various complexes were studied by using KO cells or specific inhibitors. I report here on the intervention of the class III phosphoinositide 3-kinase (PI3K) complex, ubiquitin-like conjugation systems and the endosomal system for clearance of excess/damaged ER during recovery from an acute ER stress. Moreover, I found that the ULK1

* ***Fumagalli, F., et al.*** *Nature Cell Biol. 2016, 18 : 1173-1184.*
[#]Highlights in Nature Reviews Molecular and Cellular Biology, 17
[#]Recommended by the Faculty of 1000

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complex, necessary for starvation-induced autophagy, is dispensable for recovER-phagy. All in all, I show that a non-canonical autophagy pathway ensures clearance of excess/damaged ER during the recovery phase following a transient ER stress.

Daniela Iannotta

Characterization of Antibody-Protein Interaction in Diphtheria Toxin

Supervisor: Luca Varani // Co-referee: Achim Stocker, Jürg Gertsch
Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland

Diphtheria is an extensively studied bacterial protein toxin and acts as a model for toxin infection. Despite Diphtheria Toxin (DT) being known for several decades, no structural and little biochemical information on DT/antibody interaction is available. In this project we aim to provide a structural and functional characterization of six human monoclonal antibodies (mAbs) that can potently neutralize DT, with the final goal of understanding the determinants of efficient toxin neutralization. One hypothesis is that the mAbs with higher affinity for DT are also the best neutralizers. Intriguingly, this is not the case and there is no direct correlation between binding and neutralization properties. The mAb binding site and mechanism of action are, therefore, likely to be more relevant for neutralization. Cellular infection by DT starts with the toxin binding its receptor (HB-EGF) on the cell surface. The toxin is then internalized by endocytosis. Subsequently, due to the lower endosomal pH, DT changes conformation and fuses with the endosomal membrane. DT catalytic (DT-C) domain is then translocated to the cytosol across the membrane and cleaved. The catalytic domain here exerts its toxic action by inhibiting protein synthesis. Each step of this mechanism can be potentially targeted and inhibited by an antibody.
DT is formed by three distinct structural domains and the best neutralizing mAbs bind to the receptor binding domain of the toxin (DT-R). Surprisingly, however, they do not inhibit receptor binding according to our SPR-based assays. Clearly, they must be targeting another step of the infection mechanism, which we aim to identify during the rest of my PhD work.

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Federico Mele

Molecular regulation of cytokine production and response to Mycobacterium tuberculosis by human CD4 memory T cells

Supervisor: Federica Sallusto // Co-referees: Christoph Müller, Jens Stein
University of Bern, Switzerland

In order to protect our organism from the invasion of pathogens, our immune system has developed the ability to differentiate specialized subsets of CD4+ T helper cells that recognize different classes of antigen. To better understand the T cell responses to different pathogens in humans, we first characterized the distribution of antigen-specific CD4+ T cells among different memory cell subsets. We found that the response against viruses was present predominantly in the TH1 cell compartment, while helminthes and allergens were recognized mainly by the TH2 cell compartment, and finally the T cell compartment characterized by CCR6 expression was enriched of lymphocytes specific for bacteria and fungi.

On the basis of this knowledge, in the first part of the presented work we studied the mechanistic features involved in the differentiation, maintenance and function of pathogen-specific memory T cells. Specifically, we characterized some of the underlying mechanisms of T cell activation and polarization, and we found that the phenotype that is acquired by a memory T cell during a primary response intrinsically contributes to determine the threshold of activation of this same cell during secondary antigenic exposure.

In the second part of this work, we analyzed more in details the CD4+ T cell response against one specific pathogen, namely M. tuberculosis (MTB). We found that the response against MTB was confined to a very specific subpopulation of CD4+ T cells, namely CCR6+ TH1* cells, and it was principally directed against three antigenic islands, all related to the bacterial secretion systems.

In summary, our work provides a better characterization of the overall antigen-specific responses to pathogens in primary human CD4+ T cells, with a detailed analysis of the immune responses against MTB, as well as the identification of some of the molecular mechanisms that may contribute to the different responses provided by each individual subset.

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Sara Montagner

Understanding mast cell proliferation and functions via epigenetic modifications and microRNAs

Supervisor: Silvia Monticelli // Co-referees: Hans-Uwe Simon, Mariusz Nowacki
University of Bern, Switzerland

Mast cells are immune cells able to modulate a number of physiological processes, as well as to orchestrate the response to pathogens and allergens, making them crucial not only in innate immunity but also in disease. Indeed, alterations in the number, localization and reactivity of mast cells are typical features of systemic mastocytosis (SM), a myeloproliferative disease characterized by an increase in mast cell burden. Although some genetic alterations specifically linked to SM have been identified, other unknown molecular mechanisms may contribute to the onset and progression of this disease. In particular, epigenetic modifications as well as microRNAs (miRNAs) have been shown to be key modulators of hematopoietic differentiation through transcriptional and post-transcriptional mechanisms of regulation of gene expression, respectively. Cytosine methylation (5mC) in the genomic DNA is a heritable epigenetic modification that can be modified by TET enzymes, which are able to oxidize 5mC to 5-hydroxymethylcytosine (5hmC) and further oxidation products. Alterations in the methylation landscape as well as mutations in the TET2 gene are implicated in the pathogenesis of a number of myeloid diseases, including SM, but the role of epigenetic mechanisms of regulation of gene expression in mast cell biology remains for the most part to be investigated.

The overall aim of my thesis is therefore the characterization of molecular mechanisms that regulate mast cell differentiation, proliferation and functions, with a particular focus on epigenetic modifications in health and disease.

Specifically, I contributed to the investigation of a molecular circuitry that comprises miR-146a and NF-κB1 in regulating mast cell homeostasis and survival, I characterized the interaction between the oncogene KIT and its regulator miR-221 in the context of SM, and I also participated to the analysis of the role of CD25 (a marker for pathogenic mast cells) in murine mast cells, which identified two new mast cell subsets that display different abilities to proliferate and produce cytokines.

While I contributed to these collaborative projects in the lab, the majority of my PhD work has been dedicated to understanding the role of DNA hydroxymethylation and TET proteins in mast cell biology and disease. Specifically, we observed significantly lower levels of 5hmC in SM patients compared to healthy controls, and we found that especially low levels of 5hmC correlated with a higher mutational load in the KIT oncogene, the most common genetic lesion in these patients, highlighting the importance of these modifications in SM and more in general in mast cell biology. We therefore investigated the role of TET2 and DNA hydroxymethylation specifically in mast cells, and we identified a cell-intrinsic delay in the differentiation capabilities of Tet2-ablated cells, together with reduced cytokine production and significant hyperproliferation, which associated with extensive changes in transcriptome and 5hmC distribution. Both differentiation and effector functions could be modulated by the enzymatic activity of the other TET family members, indicating that these phenotypes are largely under the control of compensatory mechanisms. Importantly, and potentially connecting our findings to diseases and specifically to SM, the proliferative defect could be rescued exclusively by the re-expression of the TET2 protein itself.

In conclusion, our work provides new insights about the molecular mechanisms regulating mast cell biology, both through the analysis of selected miRNAs and the investigation of epigenetic modifications in normal and diseased conditions, potentially leading to novel points of entry for either diagnosis or treatment of mast cell-related diseases.

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Lisa Perruzza

The purinergic P2X7 receptor at the interface between adaptive immune system and bacterial commensals in the intestine

Supervisor: Fabio Grassi // Co-referees: Kathy McCoy, Philippe Krebs
University of Bern, Switzerland

The P2X7 receptor is an ATP-gated nonselective cationic channel expressed in a variety of cell types. In T cells protracted receptor stimulation leads to opening of a pore permeable to molecules up to 900 Da and cell death. Mice with deletion of P2rx7 show expansion of T follicular helper (Tfh) cells in the Peyer's patches of the small intestine that results in enhanced IgA secretion and binding to commensals. The deregulated IgA response by lack of P2X7 activity in Tfh cells results in impaired glucose metabolism and increased fat deposition. This phenotype further deteriorated by providing a high-fat diet to mice, suggesting that P2X7 deficient animals are more sensitive to increased caloric intake. Notably, rederivation of mice in germ free conditions and administration of antibiotics dramatically improved the phenotype of P2rx7-/- mice, suggesting the involvement of the microbiota in the observed metabolic alterations. The causal role of microbiota in influencing the phenotype of P2rx7-/- mice was confirmed by fecal transplantation experiments. The analysis of Icos-/- and Icos-/-P2rx7-/- double knock-out mice, which are both devoid of Tfh cells, revealed undistinguishable metabolic parameters and no differences in microbiome composition demonstrating that lack of P2rx7 in Tfh cells, was responsible for the modified microbiota selection and impaired glucose metabolism we observed in P2rx7-/- mice. Adoptive transfer of WT and P2rx7-/- Tfh cells into Cd3e-/- mice further demonstrated that P2rx7-/- Tfh cells were sufficient to reproduce glucose metabolism alterations. Taxonomy of IgA coated versus uncoated intestinal bacteria and correlation of immunologic and metabolic parameters revealed an important role for P2X7 expression in Tfh cells in diversifying the T dependent IgA response in the small intestine and shaping commensals composition to promote metabolic homeostasis of the host. The results of this study suggest that extracellular ATP might constitute an inter-kingdom signaling molecule to modulate high-affinity IgA response and ensure host-microbiota mutualism.

* ***Perruzza, L., et al.*** *Cell Rep. 2017, 18:2566-2575.*

* ***Rezzonico Jost, T., et al.*** *J Leukoc Biol. 2016, 99:1077-1087.*

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Viola Puddinu

Role of the atypical chemokine receptor 3 in diffuse large B cell lymphoma

Supervisor: Marcus Thelen // Co-referees: Adrian Ochsenbein, Jens Stein
University of Bern, Switzerland

According to the current World Health Organization classification of lymphoid neoplasms, lymphomas can be divided into two main groups: the Hodgkin B-cell lymphomas and the non-Hodgkin B-cell lymphomas. In the latter case tumor transformation occurring during the germinal center reaction, results in the formation of several tumors, among them the Diffuse Large B-Cell Lymphomas (DLBCLs) accounts for 30 to 40% of all newly diagnosed lymphomas. DLBCL is a high grade aggressive neoplasm with fast course of the disease. DLBCLs are highly heterogeneous, nevertheless gene expression analysis led to the identification of the Germinal Center (GCB) like and Activated B cell (ABC) like molecular subtypes, characterized by different ontogeny and prognostic outcomes. The introduction of the monoclonal anti-CD20 antibody Rituximab in combination with standard chemotherapy (R-CHOP treatment) ameliorated the overall survival (OS) and the Progression-Free Survival (PFS) of DLBCL patients, in particular in GCB cases (45% vs 80% of 3-year OS and 40% vs 74% of 3-year PFS rate). Nonetheless patients that don't achieve a cure

with the first line gold standard treatment have still poor prognosis and new therapies are required to target refractory DLBCL cases. The atypical chemokine receptor ACKR3 (formerly CXCR7) is a seven transmembrane domain receptor (7TMDR) with a presumed GPCR-like structure which was recently identified as a novel high affinity receptor for CXCL12 and somewhat lower affinity for CXCL11. ACKR3 was shown to function as scavenger removing excess of chemokine therefore participating in the formation of chemokine gradients modulating the CXCL12/CXCR4 axis. Several studies highlighted the involvement of ACKR3 with various malignancies including breast, lung and prostate cancers. This observation led to the hypothesis that ACKR3 could be involved during lymphomagenesis and progression. We demonstrated that ACKR3 is expressed mostly in GCB DLBCL cell lines and is functional on VAL cells. Subcutaneous conditioning of VAL cells increases ACKR3 expression and activity. Genetic ablation of ACKR3 resulted with reduced CXCR4-mediated transmigration through primary murine and human lymphatic endothelial cells (LECs) in vitro. Moreover ACKR3 inhibition or genetic ablation dramatically influences lymphoma cell invasiveness resulting with reduced organ infiltration in mouse models of DLBCL. Our data indicate that ACKR3 is required for tumor spreading and tissue infiltration in DLBCL. Therefore we propose ACKR3 as promising therapeutic target for cases of ACKR3-expressing lymphomas.

Andrea Romagnani

Role of ATP-gated ionotropic P2X7 receptor in T effector memory cell physiology

Supervisors: Fabio Grassi // Co-referees: Jürg Gertsch, Marlene Wolf
University of Bern, Switzerland

Effector memory CD4 cells are generated following priming of naïve clones in secondary lymphoid organs. The majority of effector cells generated in the primary response are eliminated during the contraction phase of the immune response. A residual population of quiescent, long-lived cells will constitute the memory pool of antigen experienced CD4 cells. This pool of memory cells is distributed in two classes, namely T effector memory (TEM) cells able to migrate to inflamed peripheral tissues and provide immediate effector response, and T central memory (TCM) cells, which home to secondary lymphoid organs and differentiate to effector cells in response to antigenic stimulation. Understanding cell intrinsic programs that limit TEM cells lifespan and expansion in peripheral tissues have implications in a number of pathophysiological conditions, including autoimmunity, transplant rejection and cancer. Extracellular nucleotides are pleiotropic regulators of mammalian cell function. TEM cells are characterized by robust expression of P2rx7, which encodes the ATP-gated ionotropic P2X7 receptor. Two signaling patterns characterize P2X7 signaling that result in opposite outcomes. On one hand, saturating concentrations of ATP or prolonged stimulation results in dilation of a pore, excessive cation and water influx, and cell death. On the other hand, P2X7 stimulation at low concentrations of ATP generates small-amplitude currents that can promote cell growth and proliferation. Here we show that P2X7 activity limits TEM cells survival and expansion both in vitro and *in vivo*. TEM cells are exquisitely sensitive to P2X7 mediated cell death unless not previously stimulated via CD3 and CD28 that silence P2X7. Lack of P2X7 results in enhanced expansion of TEM cells in lymphopenic mice and upregulation of inducible T-cell costimulator (ICOS) that promotes germinal center reaction. In addition to cell death, P2X7 activity promotes transcription of cyclin-dependent kinase inhibitor 1A (Cdkn1a) encoding for p21Waf1/Cip1 and premature senescence of TEM cells. The tumor microenvironment is reach in extracellular ATP that affects TEM cells survival and function. However, lack of P2X7 in TEM cells promoted expansion of pro-inflammatory CD4 cells infiltrating melanoma and tumor regression via enhanced

***Rezzonico Jost, T., et al.* ***
J Leukoc Biol. 2016, 99:1077-1087.

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activation of CD8 cytotoxic cells. We hypothesize P2X7 constitutes a checkpoint for CD4 TEM cells to limit expansion of cells with uncontrolled specificity in inflammatory environments.

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Joshua Hoong Yu Tan

Investigation of cross-reactive antibodies against Plasmodium falciparum-infected erythrocytes

Supervisors: Antonio Lanzavecchia // Co-referees: Peter Bull, Kevin Marsh
University of Oxford, United Kingdom

Cross-reactive antibodies have recently come into focus as promising tools for targeted vaccine design in the fields of HIV and influenza, but the identification of such antibodies that bind to Plasmodium falciparum-infected erythrocytes has proven difficult due to extensive clonal antigenic variation on the surface of these cells. In this study, I report the identification of cross-reactive human monoclonal antibodies that bind to P. falciparum-infected erythrocytes from most parasite strains tested. These antibodies gained broad reactivity through the insertion of a large piece of DNA between the V and DJ segments of the antibody heavy chain, which is encoded on chromosome 14. The core of the insert, which is the primary element required for binding to the infected erythrocytes, codes for the collagen-binding domain of LAIR1, an immunoglobulin superfamily inhibitory receptor that is encoded on chromosome 19. In each of the donors studied, the LAIR1-containing antibodies were derived from a single B cell clone and acquired mutations that increased binding to infected erythrocytes and reduced binding to collagen. The targets of these antibodies were identified as members of the RIFIN variant surface antigen family. The LAIR1-containing antibodies were able to opsonize and agglutinate P. falciparum-infected erythrocytes, suggesting that they may be useful in reducing parasite burden *in vivo*. Using newly developed assays, I show that the presence of LAIR1-containing antibodies is not limited to a particular place or population, but can be found quite commonly in individuals from different malaria-endemic areas. These findings demonstrate a novel pathway of antibody diversification by interchromosomal DNA transposition that results in the production of functional antibodies and suggest that RIFIN epitopes that are recognized by the LAIR1-containing antibodies may be potential candidates for the development of a blood-stage malaria vaccine.

* ***Tan, J., et al.*** *Nature. 2016, 529:105-109.*

Tomasz Wypych

The role of B cells as antigen presenting cells in a mouse model of asthma

Supervisors: Fedrica Sallusto // Co-referees: Jens Stein, Kathleen McCoy
University of Bern, Switzerland

Allergy is a major problem in industrialized countries nowadays and its prevalence is on the rise. For example, according to the World Health Organization, asthma is estimated to affect around 300 million people worldwide with 250 000 deaths per year due to the disease. By 2025, its prevalence is predicted to increase by more than 100 million.

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 CD4+ T cell activation during primary or secondary response to allergens. Using mouse model of asthma, we investigated the above issues using house dust mite extract (HDM) derived from Dermatophagoides pteronyssinus, a clinically relevant allergen. We observed that B cells serve as potent antigen presenting cells (APCs) in vitro and restimulate *in vivo*-primed HDM-specific T helper cells. Interestingly, they acquired HDM antigens independently of the B cell receptor (BCR) specificity. This explains their efficacy in our model since HDM uptake and antigen presentation to CD4+ T cells was not restricted to rare Ag-specific B cells. Finally, by depleting B lymphocytes before HDM challenge, we observed a pronounced reduction in the allergic response manifested by reduced numbers of effector/memory T helper cells, eosinophils and neutrophils in the lungs as well as impaired capacity of total lung cells and lung effector/memory T helper cells to secrete Th2-associated cytokines upon ex-vivo restimulation.

Using HDM model of allergic sensitization, we also investigated the role of B cells in in the induction of HDM-specific CD4+ T cell responses. By employing two different approaches: B cell depletion before priming and sensitization of transgenic mouse strain in which MHC-II expression is restricted to B cell lineage (hereafter referred to as the B-MHC-II strain), we dissected necessity of B cells to prime T helper cell responses from their capacity to do so. Using the latter approach, we were also able to assess if B cells may initiate CD4+ T cell differentiation from naive precursors or their role would be restricted to amplification of DC-initiated pool of effector cells still residing in the lymph node. We found that depletion of B cells before HDM sensitization resulted in impaired secretion of IFN-γ, but not of Th2-associated cytokines by CD4+ T cells after ex-vivo restimulation. This suggested that B lymphocytes may be important for priming of Th1 rather than Th2 cells. Sensitization of the B-MHC-II strain of mice revealed that B cells are capable of initiating Th1 and Th17 but not Th2 cell differentiation from naive CD4+ T cell precursors.

These data reveal new mechanisms leading to initiation and exacerbation of allergic response and may have implications for designing new therapeutic strategies to combat house dust mite allergy.

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SECTION 4

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John Skehel
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Kathleen McCoy
"Microbial colonisation early in life impacts innate and adaptive immune regulation"
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Burkhard Becher
"How T cells instruct myeloid cells in autoimmunity"
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Elena Zagato
"Crosstalk between the intestinal microbiota and the host in health and disease"
Department of Experimental Oncology, European Institute of Oncology (IEO), Milan (IT) / 15.10.2015

Fabio Santoro
"Epigenetic Therapy: The Unfulfilled Promise"
Department of Experimental Oncology, European Institute of Oncology (IEO), Milan (IT) / 23.10.2015

Sai Reddy
"Accurate antibody repertoire sequencing by molecular amplification fingerprinting"
Department of Biosystems Science and Engineering (D-BSSE), ETH Zürich, Basel (CH) / 29.10.2015

Claudio Peri
"A computational biology approach for epitope discovery, epitope design and the study of protein-protein interactions"
Computational Biology Unit, Istituto di Chimica del Riconoscimento Molecolare (CNR-ICRM), Milan (IT) / 11.11.2015

Luigia Pace
"Linking heterochromatin architecture and CD8+ T cell programming"
Immunity and Cancer Unit, Institut Curie Paris (FR) / 15.12.2015

Seminar Programme 2016

Oreste Acuto
"T cell receptor signalling: triggering, regulation and long term effects"
Sir William Dunn School of Pathology, University of Oxford, Oxford (UK) / 18.01.2016

Petr Cejka
"Making the cut: DNA end resection initiates DNA double-strand break repair by homologous recombination"
Institute of Molecular Cancer Research, University of Zurich, Zurich (CH) / 19.01.2016

Felix Meissner
"The Social Network of Immune Cells: A Proteomics Perspective"
Max Planck Institute of Biochemistry, Martinsried (DE) / 02.02.2016

Martin Bachmann
"Immunological basis for therapeutic vaccines based on induction of antibody responses"
Jenner Institute, University of Oxford, Oxford (UK) / 05.02.2016

Olivier Dillenseger
"From breeder to user: two different lives?"
Janvier Labs, Saint-Berthevin (FR) / 11.02.2016

Hans-Georg Rammensee
"Development of individualized antigen-specific cancer immunotherapy"
Department of Immunology, University of Tübingen, Tübingen (DE) / 16.02.2016

Albert Bendelac
"Shared Pathways of Innate and Innate-like Lymphocyte Development"
Department of Pathology, The University of Chicago, Chicago (US) / 11.03.2016

Oliver Zerbe
"Protein NMR at the University of Zurich"
Department of Chemistry, University of Zurich, Zurich (CH) / 15.03.2016

Francesco Di Virgilio
"The strange case of Dr Jekyll and Mr Hyde: the dual role of the P2X7 receptor"
Oncology and Experimental Biology, University of Ferrara, Ferrara (IT) / 14.04.2016

Dorianna Sandonà
"Life, death and repair of alpha-sarcoglycan"
Department of Biomedical Sciences, University of Padova, Padua (IT) / 15.04.2016

Petr Broz
"Molecular mechanisms of inflammasome assembly and signalling"
Focal Area Infection Biology, Biozentrum, University of Basel, Basel (CH) / 22.04.2016

Paola Picotti
"Probing protein structural changes in health and disease"
Institute of Biochemistry, Department of Biology, ETH Zürich, Zurich (CH) / 03.05.2016

Bernd Bodenmiller
"Highly multiplexed imaging of tumor tissues by mass cytometry for precision medicine applications"
Institute of Molecular Life Sciences, University of Zurich, Zurich (CH) / 03.05.2016

Thomas Lemmin
"Insights into the dynamics of the HIV-1 Env glycan shield"
Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco (US) / 20.05.2016

Benoît Kornmann
"Interorganelle communication: new approaches for new questions"
Institute of Biochemistry, Department of Biology, ETH Zürich, Zürich (CH) / 15.07.2016

Davide Robbiani
"DNA recombination in lymphocytes"
Browne Center for Immunology, Rockefeller University, New York (US) / 22.07.2016

Federico Iovino
"Crossing the barrier: how bacterial pathogens invade the brain"
Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm (SE) / 05.09.2016

Milos Matkovic
"Molecular Basis of Biased Signaling in the Angiotensin II Type 1 Receptor"
Laboratory of Biomolecular Research, Paul Scherrer Institut, Villigen (CH) / 20.09.2016

Claudio Ciferri
"Development of a subunit-based Human Cytomegalovirus (HCMV) vaccine"
Department of Structural Biology, Genentech, Inc. South San Francisco (US) / 22.09.2016

Lee-Ann Allen
"Manipulation of neutrophil function and lifespan by Francisella tularensis"
Departments of Internal Medicine and Microbiology, University of Iowa, Coralville (US) / 23.09.2016

Michael Hannus
"RNAi in the days of CRISPR: Making optimal use of a great technology"
siTOOLs Biotech GmbH, Planegg, Martinsried (DE) / 05.10.2016

Bernd Wollscheid
"Decoding Ligand Receptor Interactions"
Institute of Molecular Systems Biology, Department of Biology, ETH Zürich, Zürich (CH) / 05.10.2016

Greta Guarda
"Novel mechanisms regulating cytotoxic responses"
Department of Biochemistry, Faculty of Biology and Medicine, University of Lausanne, Lausanne (CH) / 14.11.2016

Reinhold Förster
"How lymph-derived immune cells home to lymph nodes"
Institute of Immunology, Hannover Medical School, Hannover (DE) / 23.11.2016

Carmine Settembre
"Roles, regulators and therapeutic modulation of cellular catabolism in the skeleton"
Dulbecco Telethon Institute and Telethon Institute of Genetics and Medicine, Pozzuoli (IT) / 30.11.2016

Mario Leonardo Squadrito
''Exploiting genetic engineering of tumor-associated macrophages to promote anti-tumor responses''
Angiogenesis and Tumor Microenvironment Laboratory, Swiss Federal Institute of Technology Lausanne, Lausanne (CH) / 02.12.2016

Thomas Duhen
"Functional diversification of human T cells in health and disease"
AgonOx, Portland (US) / 12.12.2016

PEOPLE & FINANCES

SECTION 5

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IRB PERSONNEL

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Financial Data 2016 (in Swiss Francs) / *Dati finanziari 2016 (in Franchi svizzeri)*

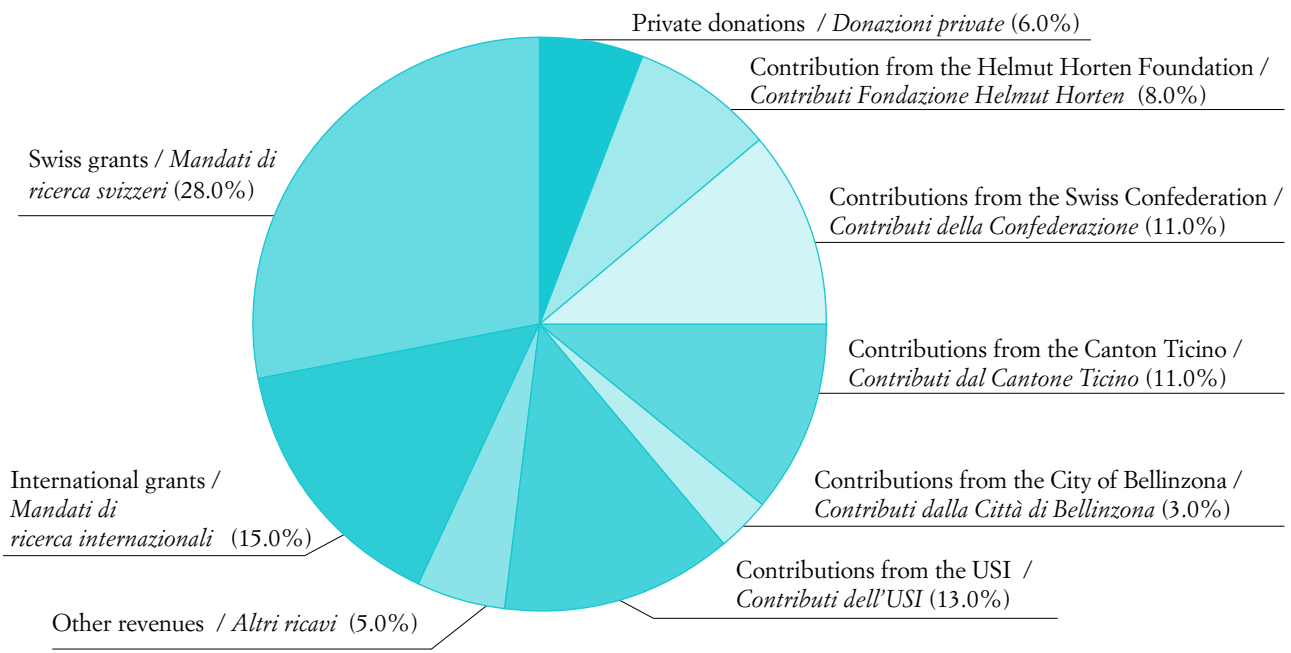
Thanks to special donations from the City of Bellinzona (CHF 2.8 million), from the Helmut Horten Foundation (CHF 1 million) and from the IRB's own endowment (CHF 0.5 million), in 2016 a fund of CHF 4.3 million was created for the financing of the construction of the new headquarters of the IRB.

In the course of the 2016 fiscal year, research activity was increased further, from CHF 7.8 million in 2015 to almost CHF 8.4 million, claiming a share of 42% of the entire budget of the Foundation.

Grazie alle donazioni straordinarie della Città di Bellinzona (CHF 2,8 mio), della Fondazione Helmut Horten (CHF 1 mio) e a una dotazione propria (CHF 0,5 mio), nel 2016 è stato costituito un Fondo di CHF 4,3 mio per il finanziamento della costruzione della nuova sede dell'IRB.

Nel corso dell'esercizio 2016 è stata ulteriormente incrementata l'attività di ricerca, passata dai CHF 7,8 mio del 2015 a quasi CHF 8,4 mio, confermando la quota del 42% dell'intero budget della Fondazione.

Funding by source 2016 / *Contributi per fonte 2016*



Balance Sheet as of December 31, 2016 (In Swiss Francs)
Bilancio al 31 dicembre 2016 (in Franchi svizzeri)

ASSETS / *ATTIVO*		31.12.2016	31.12.2015
1.	Liquidity / *Liquidità*	18'391'490	17'956'874
2.	Receivables / *Crediti*	1'093'014	1'376'484
3.	Temporary Receivables / *Transitori attivi*	4'203'322	3'400'160
	Current Assets / *Attivo circolante*	23'687'826	22'733'518
4.	Participations / *Partecipazioni*	12'500	12'500
5.	Financial assets / *Immobilizzi finanziari*	10'803	0
6.	Buildings / *Immobilizzi*	3'895'749	1'847'440
7.	Other fixed assets / *Altri immobilizzi*	1	1
	Fixed Assets / *Attivo fisso*	3'919'053	1'859'941
Total Assets / *Totale attivo*		27'606'879	24'593'459

LIABILITIES / *PASSIVO*		31.12.2016	31.12.2015
1.	Payables for goods and services / *Debiti per forniture e prestazioni*	733'128	1'211'338
2.	Funds for Research Projects / *Fondi progetti di ricerca*	4'764'316	4'489'515
3.	Funds for Laboratories / *Fondi dei laboratori*	5'239'738	3'780'216
4.	Various Funds / *Fondi diversi*	1'112'014	1'314'682
5.	Fund for New Building/ *Fondo Nuovo IRB*	4'300'000	0
6.	Accruals / *Accantonamenti e transitori passivi*	928'863	676'446
	Current Liabilities / *Capitale di terzi a breve termine*	17'078'059	11'472'197
7.	Long Term Loans / *Prestiti a lungo termine*	0	2'800'000
	Long Term Liabilities / *Capitale estraneo a lungo termine*	0	2'800'000
8.	Capital Resources / *Capitale di dotazione*	7'621'262	7'569'467
9.	Strategic Fund / *Fondo Strategico*	2'700'000	2'700'000
10.	Annual Result / *Risultato d'esercizio*	207'558	51'795
	Equity of the Foundation / *Capitale della Fondazione*	10'528'820	10'321'262
Total Liabilities / *Totale passivo*		27'606'879	24'593'459

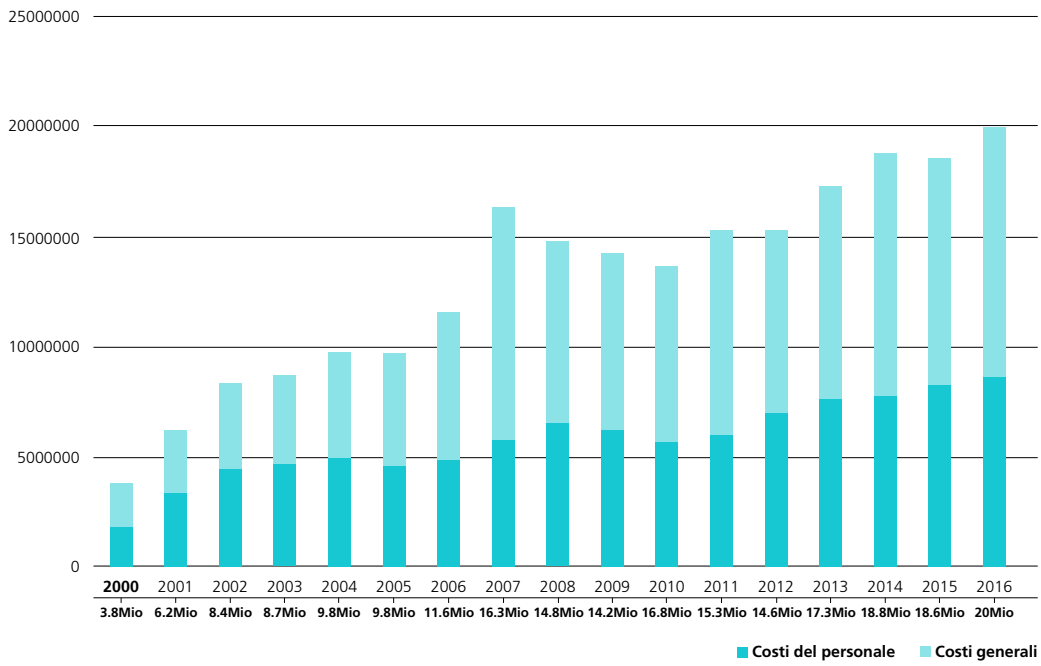
Profit and Loss Account for the year 2016 (In Swiss Francs) / *Conto economico esercizio 2016 (in Franchi svizzeri)*

REVENUES / *RICAVI*		2016	2015
1.	Contributions from the Confederation / *Contributi Confederazione*	2'128'400	2'056'350
2.	Contributions from USI / *Contributi USI*	2'650'600	2'605'853
3.	Contribution from the Canton Ticino / *Contributi Canton Ticino*	2'222'000	2'070'490
4.	Contribution from the City of Bellinzona / *Contributi Città di Bellinzona*	681'000	681'000
5.	Contributions from the Helmut Horten Foundation / *Contributi Fondazione Helmut Horten*	1'500'000	1'500'000
6.	Other Contributions / *Altri Contributi*	1'227'498	525'000
7.	Research Projects / *Progetti di ricerca*	8'369'260	7'798'118
8.	Overheads projects / *Overheads progetti*	671'513	588'039
9.	Other Revenues / *Altri ricavi*	690'366	795'575
Total Revenues / *Totale ricavi d'esercizio*		20'140'637	18'620'425

COSTS / *COSTI*			
1.	Personnel Costs / *Costi del personale*	8'695'979	8'305'505
2.	Consumables / *Materiale di consumo*	2'269'204	2'258'875
3.	Rent and Related Costs / *Affitti e altri costi dei locali*	1'528'038	1'485'911
4.	Maintenance of Buildings and Equipment / *Manutenzione immobili e attrezzature*	746'143	620'000
5.	Investments / *Investimenti*	2'235'457	834'230
6.	Administrative Costs and Various / *Costi generali amministrativi e diversi*	880'091	785'331
7.	Travels, Congresses and Guests / *Trasferte, congressi, viaggi e ospiti*	351'845	426'178
8.	Various Costs for Research / *Altri costi di ricerca*	2'311'755	1'664'041
Total operational costs / *Totale costi d'esercizio*		19'018'512	16'380'071

Margin before depreciation, amortisation and non operational items / *Risultato d'esercizio prima di ammortamenti e risultato accessorio*	1'122'125	2'240'354
Amortizations / *Ammortamenti*	500'000	998'713
Operating result / *Risultato operativo*	622'125	1'241'641
Incremento Fondi / Fund increase	3'800'000	1'300'000
Extraordinary contributions for New Building / *Contributi straordinari per Nuovo IRB*	-3'800'000	0
Total non operational and financial items and estraordinary costs *Risultato Accessorio e costi straordinari*	414'567	-110'154
Total non operational and financial items / *Risultato Accessorio*	414'567	1'189'846
ANNUAL RESULT / RISULTATO D'ESERCIZIO	**207'558**	**51'795**

Cost movements from 2000 to 2016
Evoluzione costi dal 2000 al 2016



Nella tabella allegata è riassunta l'evoluzione dei costi della Fondazione dall'inizio dell'attività nell'anno 2000, con un consuntivo di CHF 3,8 mio, all'anno 2016 con CHF 20 mio. Nel medesimo periodo i costi del personale sono aumentati da CHF 1,8 milioni a CHF 8.7 milioni, e tutti gli altri costi d'esercizio da CHF 2 milioni a oltre CHF 11 milioni.

The attached table shows the summary of cost movements of the Foundation starting from the beginning of its activity in the year 2000, with a final balance of CHF 3.8 million, up until 2016 with a final balance of CHF 20 million. In the same period, the personnel costs were increased from CHF 1.8 million to CHF 8.7 million, and all the other operating costs from CHF 2 million to more than CHF 11 million.

PUBLICATIONS
SECTION 6

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

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. 2015; 41:79-89.

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.

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Martinez-Martin, N., A. Viejo-Borbolla, R. Martin, S. Blanco, J. L. Benovic, M. Thelen and A. Alcami
Nat Commun. 2015; 6:6163.

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Beltrandi, M., D. Blocquel, J. Erales, P. Barbier, A. * Cavalli and S. Longhi
Virology. 2015; 477:42-55.

412. **Reduced DNA methylation and hydroxymethylation in patients with systemic mastocytosis.**
Leoni, C., S. Montagner, L. Deho, R. D'Antuono, G. De Matteis, A. V. Marzano, S. Merante, E. M. Orlandi, R. Zanotti and S. Monticelli
Eur J Haematol. 2015; 95:566-575.

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; 26:1532-1542.

414. **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. 2016; 13:47-56.

415. **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.

416. **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; 28:393-400.

417. **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.

418. **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; 33:2086-2095.

419. **Hitting the right spot: Mechanism of action of OPB-31121, a novel and potent inhibitor of the Signal Transducer and Activator of Transcription 3 (STAT3).**
Brambilla, L., D. Genini, E. Laurini, J. Merulla, L. Perez, M. Fermeglia, G. M. Carbone, S. Pricl and C. V. Catapano
Mol Oncol. 2015; 9:1194-1206.

420. **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.

421. **Molecular Dynamics of Biomolecules through Direct Analysis of Dipolar Couplings.**
Olsson, S., D. Ekonomiuk, J. Sgrignani and A. Cavalli
J Am Chem Soc. 2015; 137:6270-6278.

422. **An atypical addition to the chemokine receptor nomenclature: IUPHAR Review 15.**
Bachelerie, F., G. J. Graham, M. Locati, A. Mantovani, P. M. Murphy, R. Nibbs, A. Rot, S. Sozzani and M. Thelen
Br J Pharmacol. 2015; 172:3945-3949.

423. **Analysis of the performance of the CHESHIRE and YAPP methods at CASD-NMR round 3.**
Cavalli, A. and M. Vendruscolo
J Biomol NMR. 2015; 62:503-509.

424. **Structural Repertoire of HIV-1-Neutralizing Antibodies Targeting the CD4 Supersite in 14 Donors.**
Zhou, T., R. M. Lynch, L. Chen, P. Acharya, X. Wu, N. A. Doria-Rose, M. G. Joyce, D. Lingwood, C. Soto, R. T. Bailer, M. J. Ernandes, R. Kong, N. S. Longo, M. K. Louder, K. McKee, S. O'Dell, S. D. Schmidt, L. Tran, Z. Yang, A. Druz, T. S. Luongo, S. Moquin, S. Srivatsan, Y. Yang, B. Zhang, A. Zheng, M. Pancera, T. Kirys, I. S. Georgiev, T. Gindin, H. P. Peng, A. S. Yang, N. C. S. Program, J. C. Mullikin, M. D. Gray, L. Stamatatos, D. R. Burton, W. C. Koff, M. S. Cohen, B. F. Haynes, J. P. Casazza, M. Connors, D. Corti, A. Lanzavecchia, Q. J. Sattentau, R. A. Weiss, A. P. West, Jr., P. J. Bjorkman, J. F. Scheid, M. C. Nussenzweig, L. Shapiro, J. R. Mascola and P. D. Kwong
Cell. 2015; 161:1280-1292.

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Sgrignani, J. and A. Magistrato
ACS Catal. 2015; 5:3864-3875.

426. **Glycoprotein maturation and quality control.**
Molinari, M. and D. N. Hebert
Semin Cell Dev Biol. 2015; 41:70.

427. **Dynamic binding mode of a Synaptotagmin-1-SNARE complex in solution.**
Brewer, K. D., T. Bacaj, A. Cavalli, C. Camilloni, J. D. Swarbrick, J. Liu, A. Zhou, P. Zhou, N. Barlow, J. Xu, A. B. Seven, E. A. Prinslow, R. Voleti, D. Haussinger, A. M. Bonvin, D. R. Tomchick, M. Vendruscolo, B. Graham, T. C. Sudhof and J. Rizo
Nat Struct Mol Biol. 2015; 22:555-564.

428. **Neutralization and clearance of GM-CSF by autoantibodies in pulmonary alveolar proteinosis.**
Piccoli, L., I. Campo, C. S. Fregni, B. M. Rodriguez, A. Minola, F. Sallusto, M. Luisetti, D. Corti and A. Lanzavecchia
Nat Commun. 2015; 6:7375.

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