Università
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italiana

**Institute
for
Research
in
Biomedicine**

IRB Scientific Report 2018

Content

This scientific report covers the 2017-2018 research activities of the Institute for Research in Biomedicine (IRB)

Foreword
Gabriele Gendotti

The Institute for Research in Biomedicine (IRB), a university-level institute affiliated to Università della Svizzera italiana (USI) and located in Bellinzona, once again in 2018 stood out for its intense activity, which allowed it to consolidate its global presence in the field of basic research.

Thanks to internationally renowned leadership and high quality research activities, the IRB has again managed to obtain important grants in the field of competitive research and to maintain a constant presence in a network of leading collaborations.

The Institute, which now has 13 laboratories, with the same number of Group Leaders, can continue to stand out for the ideal situation that defines it, conducting high-level scientific research with limited teaching activity.

The Foundation Council was able to recruit two new members of clear fame and great experience, Prof. Rudolf Aebersold, Emeritus Professor of the ETH Zurich, and Prof. Nouria Hernandez, Rector of the University of Lausanne.

In the course of the year, close cooperation with the Faculty of Biomedical Sciences at USI was strengthened. After having appointed IRB Director Antonio Lanzavecchia and Group Leader Federica Sallusto both as Full Professors, the Faculty conferred the title of Associate Professor at USI to Greta Guarda and Petr Cejka, and that of Assistant Professor to Roger Geiger.

In addition, Prof. Federica Sallusto was appointed President of the European Federation of Immunological Societies (EFIS), an organization that represents 33 European immunological societies and brings together 13,000 researchers and clinicians.

Prof. Federica Sallusto was also elected member of the Swiss Research Council of the Swiss National Science Foundation (SNSF) in the biology and medicine division.

Also in the past year, Prof. Antonio Lanzavecchia has received a number of prestigious awards and recognitions at the international level, in particular:

- The Louis-Jeantet Foundation Award

Other IRB researchers have also received important prizes:

- Greta Guarda received the “Dr. Ettore Balli Award 2018”, a prize for young researchers in the field of medicine and biology;
- Sara Montagner received the Marie Heim-Vögtlin 2018 award from the Swiss National Science Foundation (SNSF) for her work on immune cell function published in various high-level scientific journals;
- Greta Guarda and Sonia Chelbi have received the Pfizer Award in the field of “infectious disease, rheumatology and immunology” for a several year research project on the

function of the RFX7 protein. The research was published in *Nature Immunology*.
Roger Geiger has also obtained an ERC Starting Grant of € 1.4 million from the European Research Council.
Also in 2018, researchers of the institute achieved a remarkable scientific production and were present in the most prestigious specialized journals with 55 publications and an impact factor (IF) of 10.7. In particular, research produced by Laurent Perez's laboratory on protein nanoparticles designed as a vaccine against human respiratory syncytial virus was published in the prestigious scientific journal *Cell*.
During 2018, the commission of specialists set up by USI continued the work for the designation, on the basis of an international call, of the new IRB director. The procedure is in its final stage and should lead to the appointment of the new director at the June session of the IRB Foundation Council. The new IRB director shall take office on July 1st, 2020.
The construction of the new IRB headquarters is also running smoothly. It has so far been possible to maintain the set deadlines and conclude the most important construction contracts, after public tenders and following the public procurement law. The budget forecasts are met and the objective of closing the construction site by the end of 2020 - early 2021, appears to be achievable.
On behalf of the Foundation Board and of all IRB researchers, we would like to renew our gratitude to our main supporters, in particular to the Helmut Horten Foundation, the Ruth & Gustav Jacob Foundation, the Mäxi Foundation and the Gelu Foundation, and also to the many other supporters and private donors who enable the Institute to continue to carry out its research under the best possible conditions – on a non-profit basis – and to train and develop young researchers by focusing on the acquisition of new knowledge, thus making an important contribution to innovation and improving the quality of life and economic and social growth.

Atty. Gabriele Gendotti
President of the IRB Foundation Council
Bellinzona, April 2019

Foreword
Antonio Lanzavecchia

The annual report of the Institute for Research in Biomedicine (IRB) contains a succinct description of the current research interest of its 13 research groups. The main topics of the research deal with the host defense against infectious agents and with the mechanisms of inflammatory and degenerative diseases.

The originality and relevance of the research carried out at the IRB are proven by the success of its researchers in obtaining competitive funding. Research costs, which make up 40% of the IRB budget, are in fact entirely covered by competitive funding, in particular from the Swiss National Science Foundation (SNSF), and from the European Union (Horizon 2020 and ERC). Currently, four IRB researchers have received grants from the ERC. In particular, Roger Geiger received an ERC-Starting grant in 2018 for a project that aims to optimize the use of lymphocytes infiltrating tumours to develop new cell therapies.

Research on the immune response to the malaria parasite has led to the discovery of extremely powerful antibodies capable of blocking the infection. These antibodies, described in a publication in *Nature Medicine*, recognize several sites on the surface protein of sporozoites, the infectious form of the mosquito-injected parasite, and can be used both for the prophylaxis of the infection as well as for the improvement of the current vaccine, which shows a poor effectiveness. In 2018, Antonio Lanzavecchia received the Louis-Jeantet Prize for Medicine for his research on the immune response against infections and its relevance for the development of vaccines and therapeutic antibodies. The total amount of the prize will allow pursuing research on a new mechanism of antibody generation, which was recently discovered in this laboratory. The monoclonal antibody mAb114, capable of neutralizing the Ebola virus, was tested against an active Ebola outbreak in Zaire and demonstrated therapeutic efficacy in a study done by the American National Institutes of Health.

Also in the field of antibodies, Luca Varani's group has designed *in silico* a bispecific antibody capable of blocking oligomerization and toxicity of prions. This result, published in the journal *PLOS Pathogens*, represents an important step towards the therapy of these rare but fatal pathology.

Research published in *Nature* by Federica Sallusto's group has revealed the mechanism that underlies narcolepsy, an enigmatic disease caused by the loss of neurons that produce the hypocretin hormone. In collaboration with Claudio Bassetti of the Department of Neurology at the University of Bern and Mauro Manconi of the Neurocentro della Svizzera Italiana, the IRB researchers have shown the existence, in patients suffering from narcolepsy, of T lymphocytes that recognize

hypocretin and that can kill directly or indirectly the neurons that produce it. This study represents an important achievement from the new Center of Medical Immunology (CIM) of the IRB.
Cell migration in the immune response remains a topic of primary interest to the IRB. In a publication in *Nature Immunology*, Federica Sallusto's laboratory defined the molecular mechanisms that regulate the ability of T cells to migrate and reside in peripheral tissues.
In another study, published in the *British Journal of Anesthesia*, the laboratory of Mariagrazia Uguccioni, in collaboration with the Ente Ospedaliero Cantonale of Bellinzona, reported that a local anesthetic, lidocaine, is able to inhibit the migration of tumour cells *in vitro*.
Laurent Perez, head of the "Protein Production Facility", has published as co-senior author two important works on the prestigious journal *Cell*. The first work, done in collaboration with Andrea Ciferri from Genentech, identifies neuropilin 2 as one of the receptors of the human cytomegalovirus. This study then completes a series of IRB publications that have clarified the biology of this important pathogen, paving the way for new therapies based on antibodies and vaccines. The second work is the result of a collaboration with the group of David Baker in Seattle and reports the production and use of protein nanoparticles as a vaccine against the human respiratory syncytial virus, a result of particular medical importance given the current lack of a vaccine against this important pathogen.
IRB research in the field of cellular biology continues to give excellent results. Maurizio Molinari's laboratory, which studies the quality control mechanisms of protein synthesis, defined, in a paper on *EMBO Journal*, the transport and degradation mechanisms of protein polymers.
Greta Guarda's laboratory has unveiled, in a work on *Nature Immunology*, the role of the transcription factor Rfx7 in the control of natural killer (NK) cells. Finally, Petr Cejka's laboratory continues the research line developed so far on the biochemical mechanisms of DNA repair, and in particular has analysed the role of the Sae2 protein phosphorylation in DNA resection in a publication on *Nature Communication*.
The protection of the intellectual property (IP) remains a priority for the IRB and its commitment in this area has been strengthened by a collaboration with Università della Svizzera italiana (USI). New patents have been filed in the field of antibodies, vaccines and metabolism. Humabs, the first start-up founded on IRB patents and now part of American Vir Biotechnology, continues to develop successfully and has moved its headquarters to the Business Center building in Bellinzona. The collaboration between Humabs and IRB

researchers has led to numerous publications in prestigious journals, demonstrating the effectiveness of the collaboration between industry and academy.
A new start-up, MicroVaccines, was created to develop oral vaccines thanks to the technology developed in the laboratory of Fabio Grassi. This technology is based on the observation that apyrase, an enzyme that degrades ATP, is able to increase the antibody response.
The IRB continues to play an important role in teaching. Our doctoral program has enabled 92 students to obtain the PhD degree at the ETH and other Swiss and European Universities. Many of our students have since embarked on successful careers in the academic or biopharmaceutical industry. Thanks to the contribution of the Gustav & Ruth Jacob Foundation, the 31 PhD students currently at the IRB have access to a program of lectures and seminars held by international experts, as well as the opportunity to present their work during the annual PhD students' retreat, which in 2018 was held in Einsiedeln, in the Canton of Schwyz. The IRB cooperates with the Swiss Federal Institutes of Technology in Zurich (ETHZ) and Lausanne (EPFL) as well as a number of Swiss universities.
In conclusion, together with the IRB members, we would like to express our gratitude to all current and past members of the Foundation Council for the success achieved in the search for funding and for the energy dedicated to planning the new building that will allow the IRB to expand and amplify its research areas. We are particularly grateful to our main sponsors: the Helmut Horten Foundation,
the City of Bellinzona, the Canton Ticino and the Swiss Confederation. Our gratitude also goes to those who support us through donations and grants. We believe that the progress and results of the Institute will reward their dedication to the advancement of science.

Antonio Lanzavecchia
Director of the IRB
Bellinzona, May 2019


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Research Groups

Andrea Cavalli
Computational structural biology

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

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Maura Garofalo, PhD student – Miloš Matković, PhD – Jacopo Sgrignani, PhD – Enrico Fassi, student

Molecular characterization of a novel class of STAT3 inhibitors

Jacopo Sgrignani and Andrea Cavalli

Transcription factors (TFs) are central nodes in multiple oncogenic signalling 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 signalling pathways for tumour initiation and progression. STAT3 drives tumour progression by promoting proliferation, survival, metabolic adaptation, tumour angiogenesis and immune tolerance and its downregulation by genetic or pharmacological means prevents or reverts tumourgenesis.

Many anticancer drugs inhibit upstream signalling 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 tumour models. Experimental results suggest, that this new class of compounds acts by promoting the formation of large aggregates of STAT3 and that the formation of this aggregates is a direct consequence of conformational changes, disruption of specific inter-domain interactions and partial unfolding of STAT3 induced by STAT3i.

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.

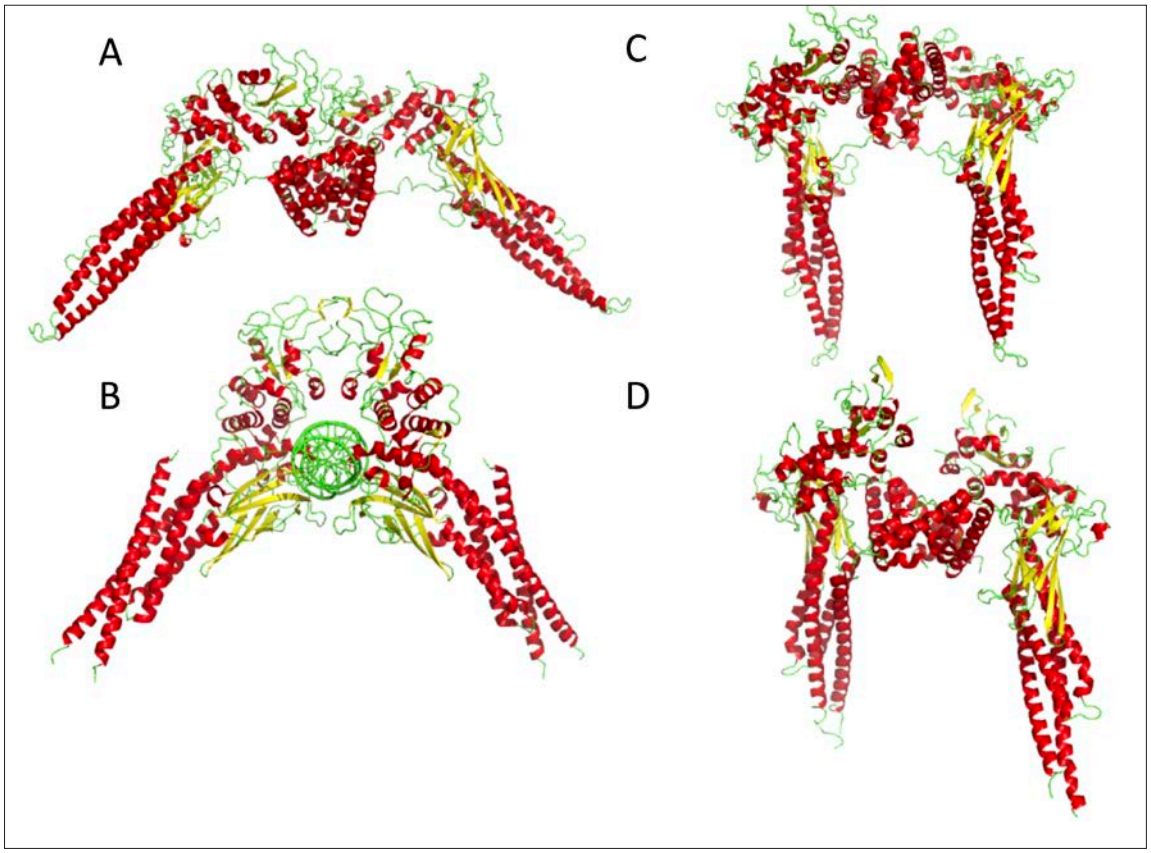

Figure 1.
Computational models of the STAT3 homodimer.

Identification of potential determinants of immunoglobulin light chain amyloidosis

Maura Garofalo and Andrea Cavalli

Immunoglobulin light chain amyloidosis (AL) is a systemic protein misfolding disease characterized by the abnormal proliferation of a plasma cell clone secreting large amount of unstable free light chains (LCs) homodimers. These latter, after misfolding are able to aggregate into toxic oligomers and amyloid fibrils, which deposit in target tissues leading to organ failure and death. Since the generation of amyloidogenic LCs is connected with antibody production, each AL patient carries a unique B cell clone secreting a different and specific LC generated by a V-J rearrangement followed by the addition of somatic hypermutations (SHM) during the process of affinity maturation. Despite several years of research, several open questions remain. In fact, it is still not understood why some free LCs are prone to amyloid aggregation while others are soluble and non-amyloidogenic, even when expressed at high concentration. Furthermore, the specific sequence and structural features responsible for toxicity of free LCs, as well as, the mechanism responsible for the conversion of these proteins in toxic species, are still unknown. Our aim is, therefore, to identify determinants of LC toxicity, by comparing toxic and non-toxic sequences.

We showed, through a statistical analysis, that toxic LCs have a significant higher chance of exhibiting somatic hypermutations as compared to non-toxic light chain sequences. Based on this finding, we devised a machine learning approach using SHM, together with structural features, as predictor variables to automatically classify previously unseen LCs sequences with a 75% precision. Next, we investigated the effect of SHM on LCs thermodynamic stability, showing that LCs homodimers are destabilized after the process of affinity maturation. Since, during the affinity maturation of antibodies LCs are always part of heterodimeric complexes with the heavy chain, we conjecture that LC-LC structures might be destabilized, and so become more aggregation prone, as a consequence of a lack of 'quality control/negative selection' for these structures.

Our results show, for the first time, that the addition of SHM in specific positions of LC sequences could be the driving force that leads to the formation of toxic species in AL disease. Or algorithm correctly identifies toxic LCs in > 75% of the cases, thus, fostering early AL-diagnosis in patients with pre-existent asymptomatic monoclonal gammopathy (MGUS) and helping reducing the high mortality of affected patients.

Design and development of inhibitors of the CXCL12/HMGB1 interaction

Jacopo Sgrignani and Mariagrazia Uguccioni

During inflammatory reactions, the production and release of chemotactic factors guide the recruitment of selective leukocyte subpopulations. HMGB1 and the chemokine CXCL12, both released in the microenvironment, form a heterocomplex, which exclusively acts on the chemokine receptor CXCR4, enhancing monocyte migration and exacerbating the immune response. An excessive cell influx at the inflammatory site can be diminished by disrupting the heterocomplex. To date, only few inhibitors of the CXCL12/HMGB1 interaction have been described. Using a computationally driven procedure we identified of a novel high affinity peptide (HBP08) able to inhibit the activity of the CXCL12 heterocomplex both on CXCR4 transfected cells and on human monocytes. HBP08 binds HMGB1 with a Kd of 0.8 ± 0.06 μM for the HMGB1/HBP08 complex, making it the strongest HMGB1 binding molecule known to date. The identification HBP08 represents an important step towards the development of innovative pharmacological tools for the treatment severe of pathological condition such as rheumatoid arthritis.

Finally, we demonstrated that a retro-inverso peptidomimetic of HBP08 conserve a good affinity for HMGB1 and that could be used to future in-vivo investigations.

Design and development of inhibitors of PDHA1 for anticancer therapy

Jacopo Srignani and Andrea Alimonti, IOR.

Cancer is now the leading cause of death in the world surpassing cardiovascular disease. No other disease destroys as many lives every year as cancer does. Furthermore, in Switzerland, prostate cancer is the second commonest malignancy and the second commonest cause of mortality among man. Although androgen deprivation therapy leads to treatment remission in 80 to 90% of treated cases, patients ultimately relapse becoming castration resistant. The treatment of castration resistant prostate cancer remains unsatisfactory. In fact, therapeutic strategies such as second-generation androgen deprivation therapies, cytotoxic agents and radionuclides still provide very limited clinical positive outcomes and the prognosis of metastatic castration resistant prostate cancer still remains poor. Thus, there are many clinical unmet in the present therapeutic options for prostate cancer. We strongly believe that, the development of novel therapies targeting the metabolic requirements of prostate cancer will constitute a major breakthrough for the therapy of this tumor. The global aim of the present research is to undertake a multidisciplinary approach to further advance the

state-of-the-art of prostate cancer treatment investigating an innovative therapeutic approach based on the inhibition of the pyruvate dehydrogenase complex (PDC) a gatekeeper regulator of the mitochondrial and lipid metabolism in cancer cells. Our best compound (FA63) has shown activity *in vitro* and *in vivo*.

Exploring the role of KIAA1199/CEMIP axis in Alport syndrome, a paediatric rare disease condition resulting in end stage renal disease

Marco Prunotto, UNIGE

Alport syndrome (AS), a paediatric rare disease caused by mutations affecting type IV collagen, a major network-forming structural component of basement membranes, is one of the best characterized Mendelian human diseases affecting 1 in 5,000-10,000 individuals. AS leads almost inevitably to loss of renal function or end-stage renal disease (ESRD). Males with the dominant X-linked (COL4A5) mutations show a severe phenotype with progression to ESRD in adolescence, early adulthood or even in childhood. Our *in vitro* and *in vivo* work has highlighted a major role for a collagen receptor tyrosine kinase called Discoidin Domain Receptor 1 (DDR1) in the onset and progression of AS. Because of the magnitude of renal damage reduction observed in DDR1 genetic deletion murine models, a series of DDR1 inhibitors have been developed by several research groups, including us. Those DDR1 inhibitors, though highly selective, have however a limited clinical value. We therefore set to explore the protective mechanism downstream of DDR1 and, in a series of experiments, we identified an enzyme, KIAA1199, selectively induced by DDR1 in presence and potentially mediating DDR1 protective role in AS.

The overall aim of the present research is to investigate the biology of DDR1/KIAA1199/HA axis in Alport syndrome both in vitro and in vivo: a) Relevance of KIAA1199 will be probed in vitro with a series of mechanistic experiments exploring the role of KIAA1199 and HA fragments in inducing cell several autonomous behaviours b) Relevance of KIAA1199 will be explored in vivo using COL4A3 KO (Alport mice) mouse model using whole or tissue specific KIAA1199 conditional KO mice, c) translational information on the role of KIAA1199 in AS will be produced generating an anti-human KIAA1199 specific and selective antibody and then using this antibody for immunohistochemistry on human tissues (in collaboration with Solange Moll, Nephrology dept., University of Geneva) and d) Molecular tools (e.g., a selective KIAA1199 inhibitor) will be developed to further investigate and characterize the DDR1/KIAA1199/HA axis both *in vitro* and *in vivo*. Potentially these molecules will then be tested in Alport mice and hopefully exploitable to protect AS patients.

Funding

Swiss Cancer League
Structural basis for the inhibition of STAT3 transcription factor by small molecules
KLS-3839-02-2016-R / 2016-2018

Swiss National Science Foundation
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310030_166472 / 2016-2019

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Research Groups

Petr Cejka
Recombination mechanisms

Petr Cejka received his Master's degree in 2000 from the Charles University in Prague and PhD in 2004 from the University of Zurich. During his PhD studies with Prof. Josef Jiricny, Petr Cejka studied the function of the mismatch repair system in human cells, as well as how mismatch repair mediates the sensitivity of DNA methylating agents used in anti-cancer therapy. 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

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Promotion of genetic diversity in meiosis: resolution of recombination intermediates

Roopesh Anand, Lepakshi Ranjha, Elda Cannavo and Petr

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 and define the regulation of its nuclease activity.

We successfully expressed and purified the human MutLγ (MLH1-MLH3) complex. We could show that MutLγ is indeed a nuclease that nicks doublestranded 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. The human MutSγ (MSH4-MSH5), also binds Holliday junctions and helps recruit MutLγ to these structures. We observed that MutSγ directly promotes the nuclease activity of MutLγ. Currently, we are testing for potential stimulatory effects of other components of the meiotic pro-crossover pathway, including EXO1.

Recombination in DNA replication: Promoting genome stability

Lepakshi Ranjha, Swagata Halder and Petr Cejka

In addition to repair double-stranded DNA breaks, homologous recombination helps to stabilize or restart replication forks in the presence of DNA damage. Specifically, in response to DNA damage, replication forks were shown to reverse, forming a 4-way structure. This helps to temporarily stabilize the fork, and prevents its breakage. Using biochemical methods, we aim to study the mechanism of replication for reversal, and define thus the functions of key proteins or protein complexes involved in this process, including RAD51, MMS222-TONSL, SMARCAL1, ZRANB3, BRCA1 and BRCA2 and the RAD51 paralogs.

Furthermore, the key homologous recombination protein RAD51 was shown to have a non-canonical function (recombination-independent) to protect stalled or reversed replication forks from degradation by MRE11 and other nuclease complexes (see below). We therefore aim to define the interplay of RAD51 with these nuclease complexes. 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 Cannavo, Anannya Acharya, Ilaria Ceppi, 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 could define how phosphorylation stimulates Sae2 and CtIP, and how this regulates the interplay with the MRX or MRX complex, respectively.

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 continue resection downstream from the MRE11-RAD50-NBS1/XRS2 complex. To this point, we study the interplay of the short-range pathway (MRN-CtIP) and the long-range pathways catalyzed by EXO1 and DNA2. 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. This includes the interplay with the single-strand DNA binding protein RPA. 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.

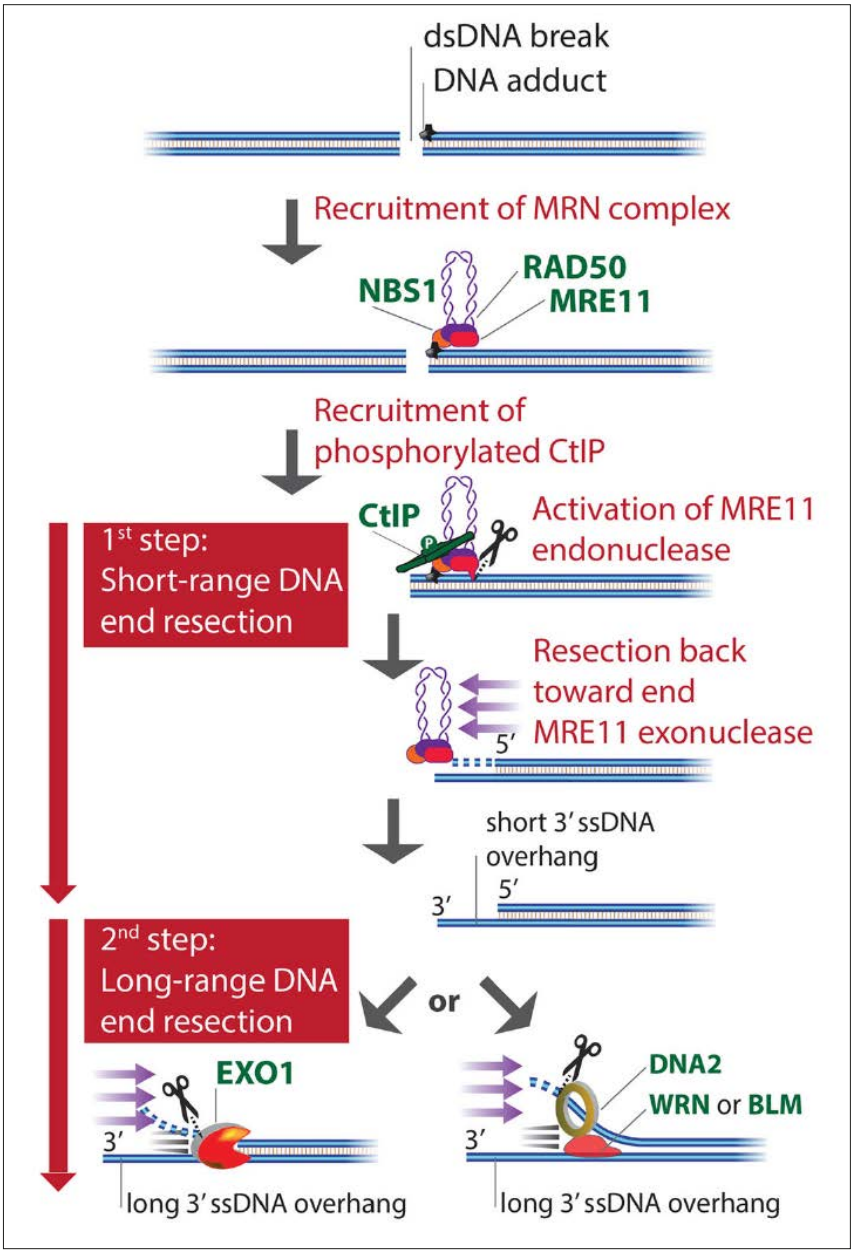

Figure 1.
Overview of DNA end resection pathways from human cells. From Ranjha et al, Chromosoma, 127:187–214, 2018.

Funding

Swiss National Science Foundation - Professorship
Repair of damaged DNA by homologous recombination and related pathways
PP00P3-159323 / 2015-2017

Swiss National Science Foundation
Beyond DNA end resection: Role of homologous recombination in the maintenance of genome stability
175444 / 2017-2021

European Research Council (ERC)
HRMECH: Nucleases in homologous recombination: from basic principles to genome editing
Horizon2020-ERC-CoG-2015 – 681630 / 2016-2021

Novartis Foundation
Investigating the mechanisms of chemoresistance in BRCA-deficient cells
2018

Helmut Horten Foundation
Structural insights into the repair of broken DNA: towards understanding the molecular basis of anticancer therapy
2017-2021

Swiss Cancer League
Investigating the mechanisms of chemoresistance in BRCA - deficient cells
KLS-4370-02-2018 / 2018-2022

Collaborations

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Publications

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Research Groups

Roger Geiger
Systems Immunology

Roger Geiger obtained his Master's degree in biochemistry from the ETH Zürich in 2007. During his PhD studies at the ETH Zürich with Ari Helenius, Roger studied how non-enveloped viruses penetrate the host cell membrane. In 2011, he joined the laboratory of Antonio Lanzavecchia at the IRB and received a transition postdoc fellowship from SystemsX. The focus of his research was on metabolic regulations during the T cell response. In 2016, Roger joined the research group of Matthias Mann at the Max Planck Institute of Biochemistry in Munich and received training in mass spectrometry-based proteomics. In August 2017, Roger started his own independent research group at the Institute for Research in Biomedicine to study T cell responses to tumours using systems biology techniques.

Research Focus

T cells are key players in the immune system with the ability to detect and eliminate infected cells and tumours. We study molecular regulations underlying T cell activation and anti-tumour activity. For this, we use a wide range of technologies including mass spectrometry-based proteomics, functional genomics, mouse models and microfluidics-based systems. Our projects aim to provide detailed insights into T cell functionality that can be translated into the clinic to improve anti-cancer immunotherapies.
We are particularly interested in the immune response to liver cancer. T cells that infiltrate liver tumours are often exhausted and do not work properly. To potentially increase their functionality, we study the underlying regulations by systematically analysing tumour-infiltrating T cells with high-resolution mass spectrometry and functional assays.
In a related project, we develop workflows to efficiently isolate T cells that recognize liver tumour antigens. Tumour-reactive T cells can be grown to large numbers and used for adoptive T cell therapies, a highly personalized form of cancer therapy. In collaboration with the research group of Andrew deMello (ETH Zürich), we use droplet-based microfluidics systems to manipulate and analyse single T cells in a high-throughput format.

Team

Group Leader:

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Members

Marco Benevento, PhD – Fernando Canale, PhD – Stefania Geiger, research assistant – Wenjie Jin, PhD student – Sivano Re – Janusz von Renesse, visiting scientist – Giada Zoppi, PhD student

Engineered non-pathogenic bacteria producing L-arginine synergize with PD-1-based cancer immunotherapy

Camilla Basso, Fernando Canale, Ning Li, Michela Perotti, Michael James, Stefania Geiger, Wenjie Jin, Jean Philippe Theurillat, Daniel Leventhal, Kip West, Jose M. Lora, Federica Sallusto, and Roger Geiger

The availability of L-arginine in tumours is a key determinant of an efficient anti-tumour T cell response. Consequently, elevation of typically low L-arginine levels within the tumour may greatly potentiate the anti-tumour responses of current immune checkpoint inhibitors, such as PD-L1 blocking antibodies. However, currently no means are available to locally increase intra-tumoural L-arginine levels. Here, we used a synthetic biology approach to develop an engineered probiotic Escherichia coli Nissle 1917 strain that colonizes tumours and continuously converts ammonia, a metabolic waste product that accumulates in tumours, into L-arginine. Colonization of tumours with these L-arginine producing bacteria elevated intra-tumoural L-arginine concentrations, increased the amount of tumour-infiltrating T cells and had striking synergistic effects with PD-L1 blocking antibodies in the clearance of tumours. The anti-tumour effect of the living therapeutic was mediated by L-arginine and was dependent on T cells. These results show that engineered microbial therapies enable metabolic modulation of the tumour microenvironment leading to enhanced efficacy of immunotherapies.

Collaborators: Camilla Basso, Michela Perotti and Federica Sallusto, IRB, Bellinzona (CH); Jean Philippe Theurillat, IOR, Bellinzona (CH); Ning Li, Michael James, Daniel Leventhal, Kip West and Jose M. Lora, Synlogic, Cambridge (USA).

Multiple mechanisms of T cell preparedness for rapid activation

Tobias Wolf, Wenjie Jin, Giada Zoppi, Matteo Pecoraro, Christopher K.E. Bleck, Tim Beltraminelli, Jan Rieckmann, Dirk Bumann, Felix Meissner, Matthias Mann, Antonio Lanzavecchia, Federica Sallusto, and Roger Geiger

Resting naïve T cells are metabolically inert but need to be prepared to quickly respond to antigens by executing a complex activation program. To investigate the molecular underpinnings of T cell homeostasis and preparedness, we quantified global protein synthesis rates and transcript abundances in resting and activated human naïve T cells. While the majority of the proteome of resting naïve T cells was stable, a small set of proteins turned over rapidly including seven transcription factors that actively maintain homeostasis and quiescence as well as the characteristic receptors IL-7R, CCR7 and CD62L. Rapid turnover enabled rapid tunability of their expression to promote activation and differentiation. Despite low glycolytic and translational rates, naïve T cells contained large numbers of glycolytic enzymes, ribosomes and mRNAs that were rapidly engaged only following stimulation to ramp up the activation program. Collectively, our study reveals a minimal maintenance program in naïve T cells and identifies protein turnover, preformed stores of enzymes and an idling translational machinery as key elements that poise T cells for rapid responsiveness.

Collaborators: Matteo Pecoraro, Jan Rieckmann, Felix Meissner and Matthias Mann, MPI, Martinsried (DE), Christopher K.E. Bleck and Dirk Bumann, Biozentrum, Basel (CH).

Funding

Roche Postdoctoral
Proteomic profiling of T cell exhaustion in periphery versus tissue in chronic infectious diseases and cancer
2018-2020

Publications

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Nat Immunol. 2017; 18:583-593.

Research Groups

Santiago F. González
Infection and immunity

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 pathogens and cancer cells 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:

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Members

Juliana Falivene, PhD – Irene Latino, PhD student – Daniel Molina Romero, PhD student – Diego Morone, Imaging Specialist – Miguel Palomino, PhD – Mauro Di Pilato, PhD – Diego Pizzagalli, PhD student – Alain Pulfer, PhD student – Tommaso Virgilio, PhD student.

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

Irene Latino 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.

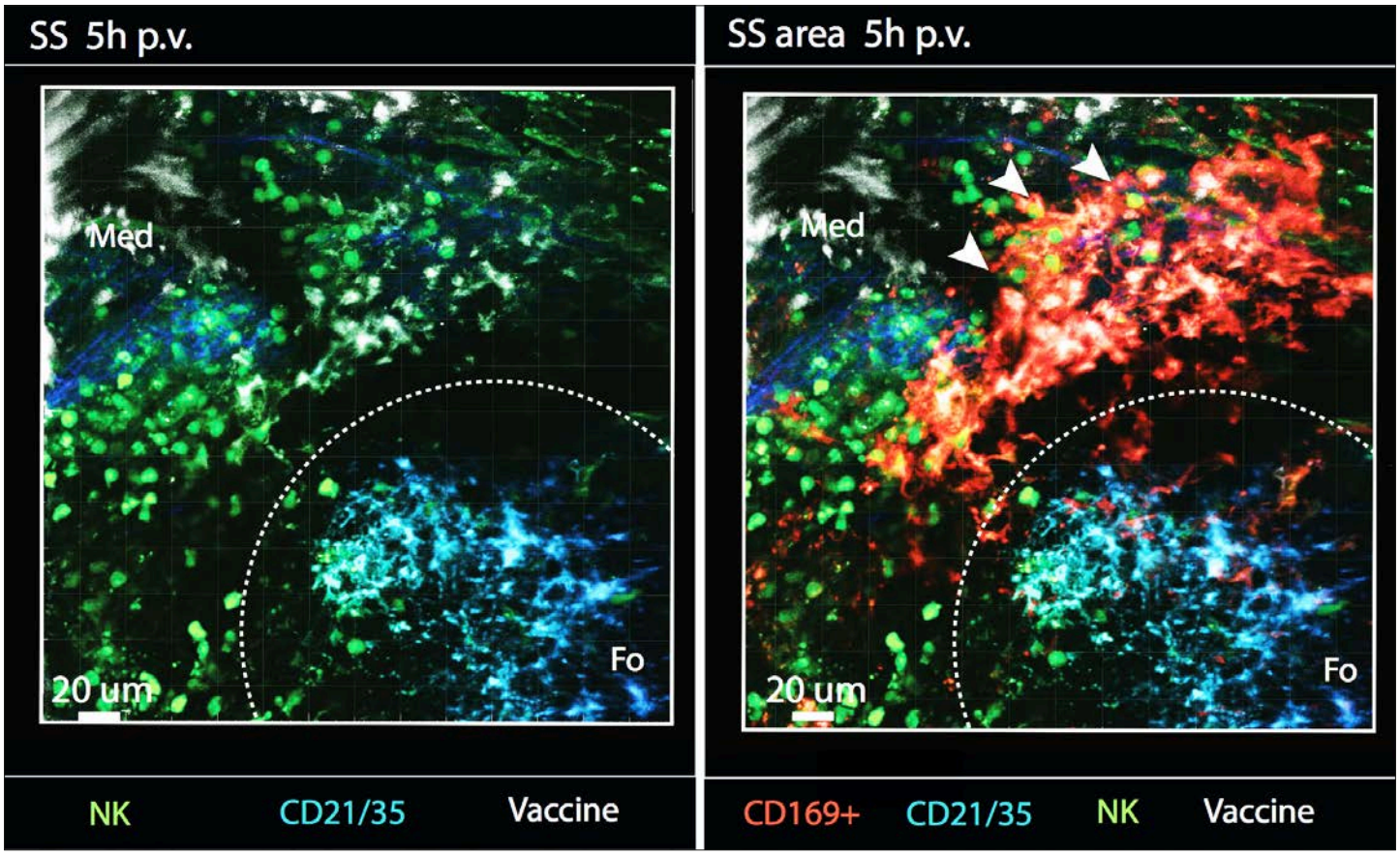

Figure 1.
2-Photon intravital micrograph showing NK cells (green) interacting with CD169+ macrophages (red) in the popliteal lymph node.

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

Diego Pizzagalli, Alain Pulfer 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, Massachussetts General Hospital, Boston (US) and Dr. Stein, University of Fribourg (CH).

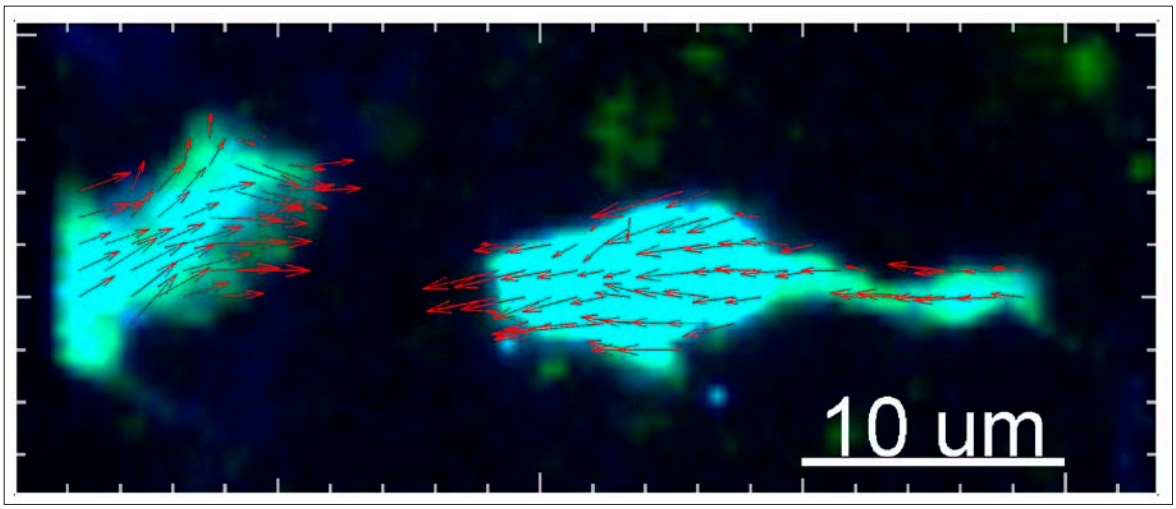

Figure 2.
Movement estimation on two fluorescently-labeled neutrophils observed by two-photon intravital microscopy. Red arrows indicate the pixel displacement.

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 expressed 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.

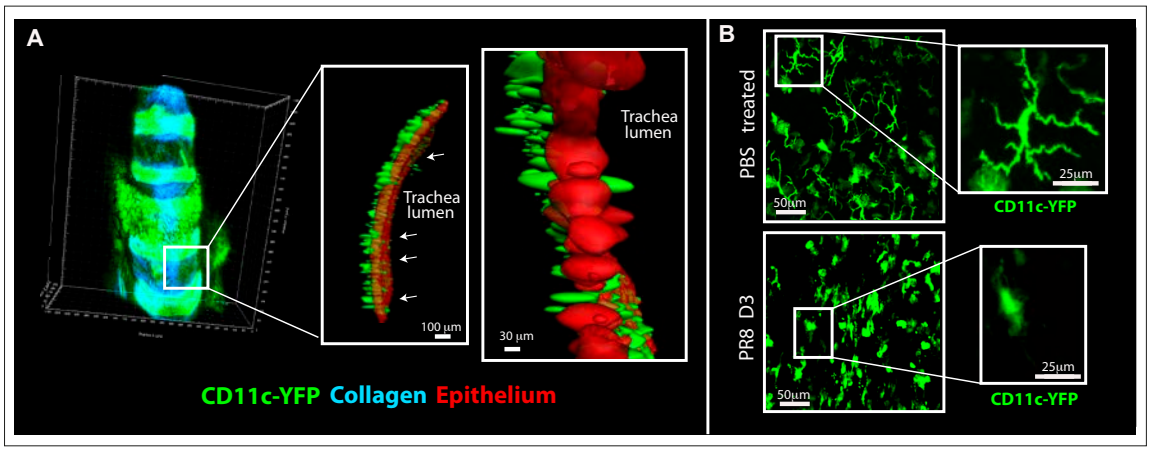

Figure 3.
A) Intravital 2-photon micrograph reconstruction showing the projection from a CD11c+ DC into the tracheal luminal space. B) Intravital 2-photon micrographs showing the changes in the morphology of the CD11c+ DC before (upper part) and after (loers part) infection with influenza virus.

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 microbiocidal 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.

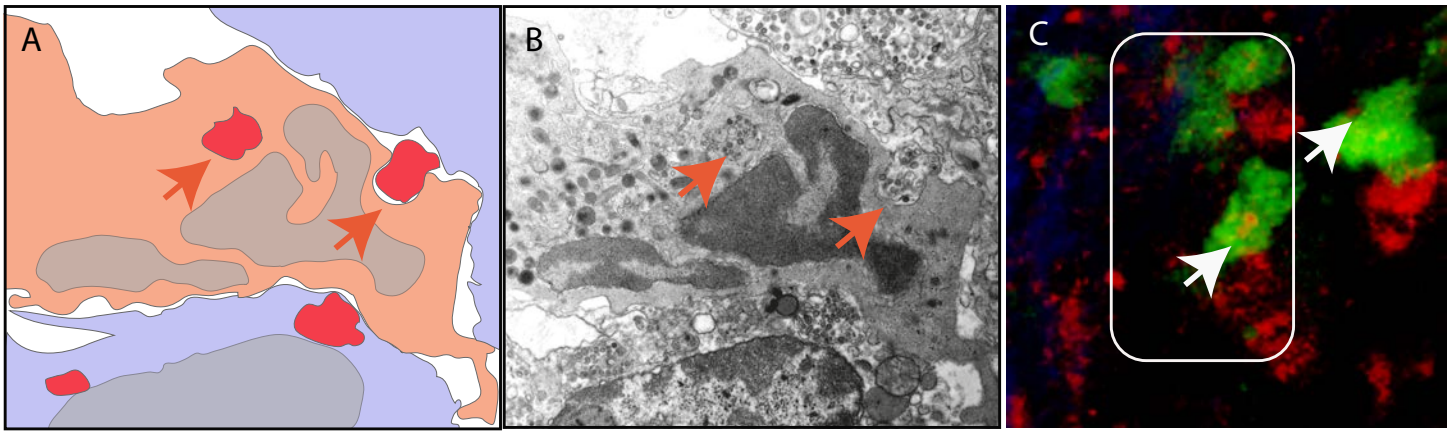

Figure 4.
A) Schematic representation of an electron micrograph (B) and 2-photon snapshot (C) showing the capture and internalication of virus (arrows) by newly recruited neutrophils.

Lymph node macrophages as initiators of the immune response

Tommaso Virgilio 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.

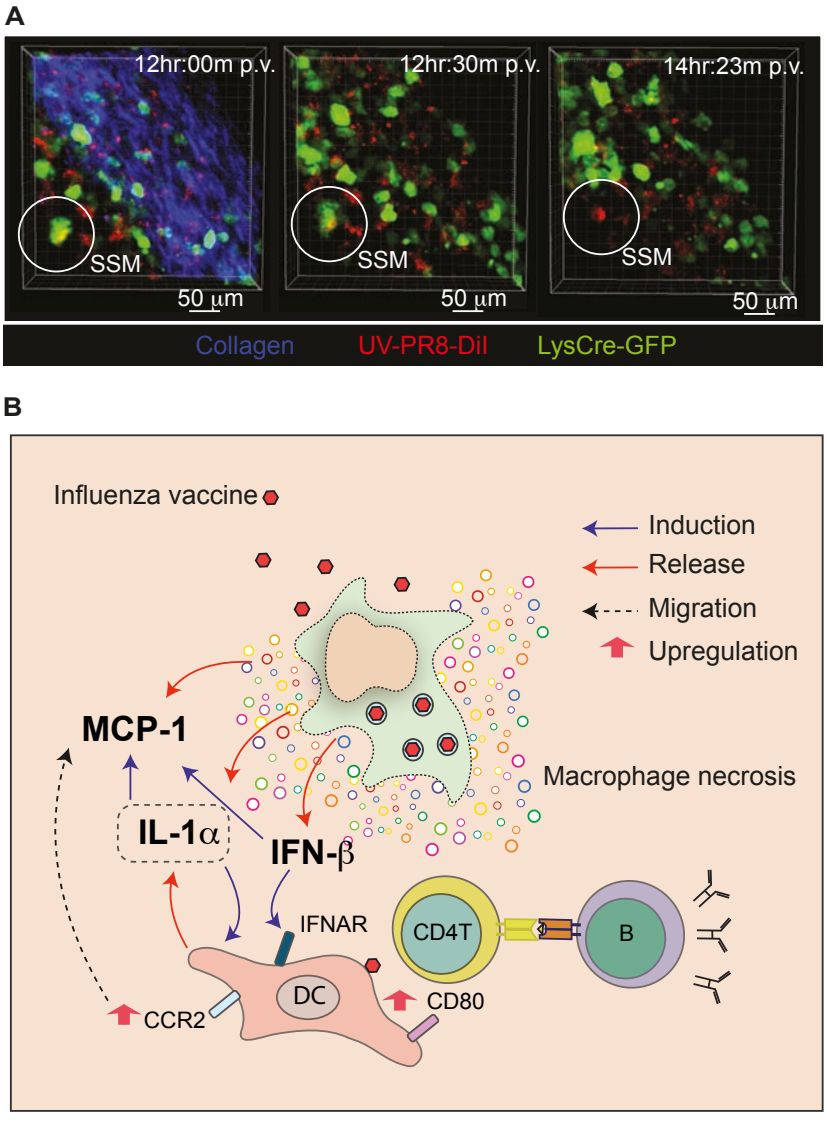

Figure 5.
A) Intravital 2PM sequence from LysMCre-GFP reporter mice (green) shows subcapsular sinus macrophages (SSM) disappearance at 12 h p.v. with DiI-labelled UV-PR8 (red) B) Proposed model of the role of SSM in the activation of dendritic cells (DC) and the initiation of the inflammatory response 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).

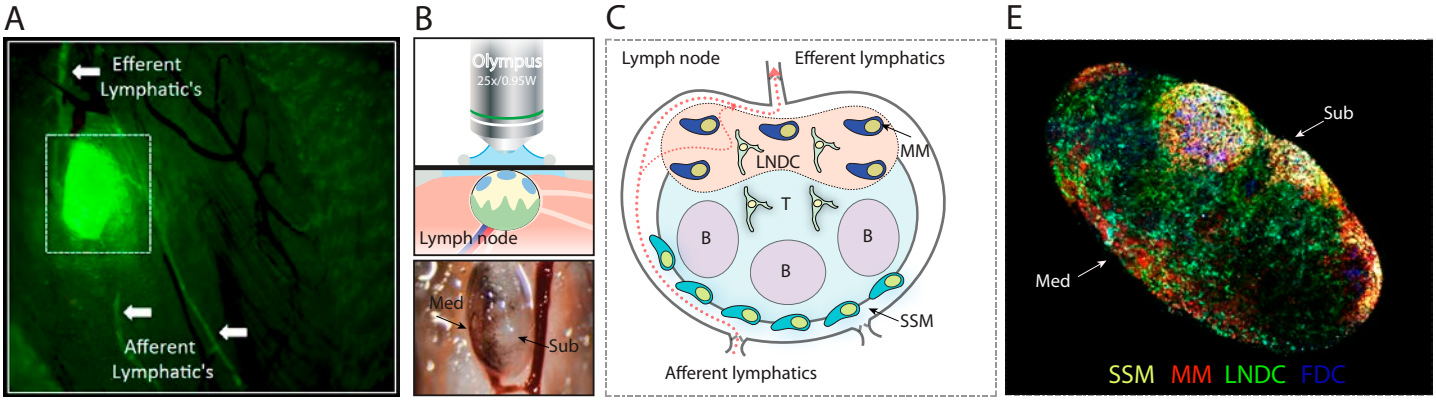

Figure 6.
A) Micrograph of a popliteal region of a Prox1-GFP transgenic mice showing the relative position of the popliteal lymph node (LN) with respect to the blood and lymphatic vessels (afferent and efferent). B) Intravital 2-photon microscopy set-up showing the microsurgery in the popliteal area. 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

Swiss National Science Foundation
Role of lymph node phagocytes in the regulation of the IgG-mediated suppression of the immune response to influenza vaccine
310030_176124 / 2017-2021

San Salvatore Foundation
Intravital study of the trafficking of B lymphoma cells in the lymphatic compartment
2018-2020

Swiss Cancer League
In vivo characterization of melanoma dissemination through the lymphatic system
KFS-4274-08-2017 / 2018-2020

European Union - Marie Skłodowska-Curie Action IF GF
TANTUMorVACCINE: Neutrophil subtypes: distinct cellular targets for therapeutic intervention
H2020-MSCA-IF-2016 / 2017-2020

European Union - Marie Skłodowska-Curie Action 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
PH-0_150369 / 2013-2017

Swiss Government Excellence Scholarship.
Study the transport of tattoo inks to the lymphatic compartment and their effect on the immune response.
2018-2019

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Publications

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Research Groups

Fabio Grassi
T cell development

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

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Members

Elisa Civanelli, Undergraduate student – Benedetta De Ponte Conti, Undergraduate student – Massimiliano Fontana, Undergraduate student – Lisa Perruzza, Post-doc – Marco Raffaelli, Undergraduate student – Tanja Rezzonico Jost, Staff scientist – Elsa Rottoli, PhD student – Matteo Raneri, Post-doc – Morena Scantamburlo, Undergraduate student – Francesco Strati, Post-doc.

Shaping of gut microbiota by secretory IgA coating conditions systemic glucose homeostasis

Lisa Perruzza, Francesco Strati and Fabio Grassi

The secretory immunoglobulin A (SIgA) in mammalian gut protects the organism from infections and contributes to host physiology by shaping microbiota composition. The mechanisms regulating the adaptive SIgA response towards gut microbes are poorly defined. Deletion of *P2rx7*, encoding for the ATP-gated ionotropic *P2X7* receptor, leads to T follicular helper (Tfh) cells expansion in the Peyer's patches (PPs) of the small intestine, enhanced germinal centre (GC) reaction and IgA secretion; the resulting alterations of the gut microbiota in turn affects host metabolism. We have defined gut microbiota modifications that correlate with deregulated SIgA secretion and metabolic alterations in *P2rx7*$^{-/-}$ mice. In particular, *Lactobacillus* showed enhanced SIgA coating in *P2rx7*$^{-/-}$ with respect to wild-type (WT) mice. The abundance of SIgA-coated lactobacilli positively correlated with Tfh cells number and body weight suggesting *Lactobacillus*-specific SIgA response conditioned host metabolism. Accordingly, oral administration of intestinal *Lactobacillus* isolates from *P2rx7*$^{-/-}$ mice to WT animals resulted in altered glucose homeostasis and fat deposition. Thus, enhanced SIgA production by P2X7 insufficiency promoted *Lactobacillus* colonization interfering with systemic metabolic homeostasis. These data indicate that P2X7 receptor-mediated regulation of commensals coating by SIgA is important in tuning the selection of bacterial taxa conditioning host metabolism.

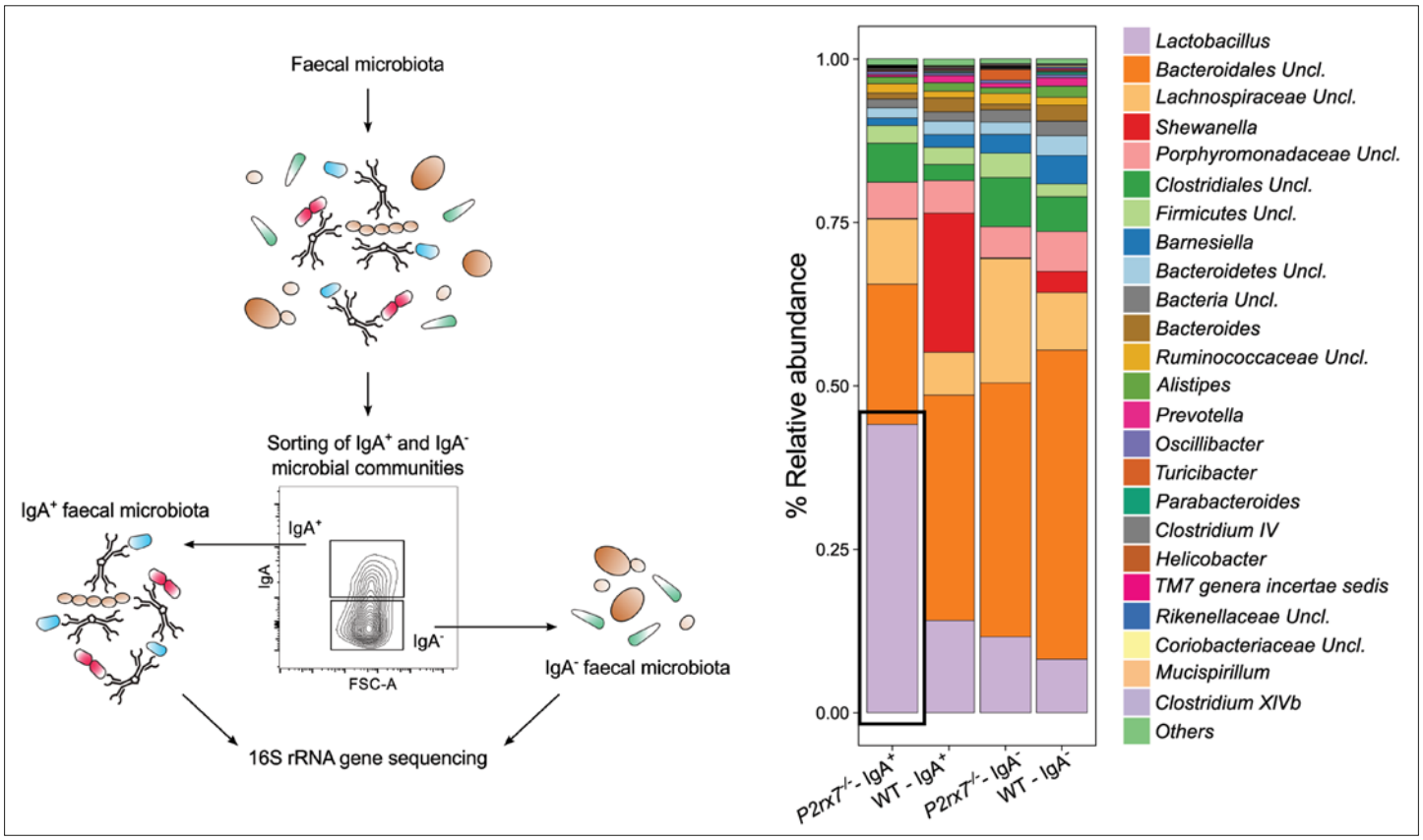

Figure 1.
Enrichment of Lactobacillus in the IgA-coated fraction of faecal microbiota from P2rx7$^{-/-}$ mice.
IgA-coated (IgA+) faecal bacteria have been separated from non IgA-coated (IgA-) counterpart by flow cytometry and characterized by 16S rRNA gene sequencing. Mean relative abundances (%), at genus level, of the IgA+ and IgA- fractions of faecal microbiota from P2rx7$^{-/-}$ and WT mice are shown. Lactobacillus representation in the IgA+ fraction from P2rx7$^{-/-}$ mice is indicated. All bacterial genera with relative abundance <0.1% are reported together and labelled as "others".

ATP released by gut colonizing bacteria as a regulator of secretory IgA response

Michele Proietti, Lisa Perruzza, Francesco Strati, Marco Raffaelli and Fabio Grassi

T cell dependent secretory IgA (SIgA) generated in the Peyer's patches (PPs) of the small intestine shapes a broadly diverse microbiota that is crucial for host physiology. The mutualistic co-evolution of host and microbes led to the relative tolerance of host's immune system towards commensal microorganisms. The ATP-gated ionotropic P2X7 receptor limits T follicular helper (Tfh) cells expansion and germinal center (GC) reaction in the PPs. Here we show that transient depletion of intestinal ATP can dramatically improve high-affinity IgA response against both live and inactivated oral vaccines. Ectopic expression of Shigella flexneri periplasmic ATP-diphosphohydrolase (apyrase) abolishes ATP release by bacteria and improves the specific IgA response against live oral vaccines. Antibody responses primed in the absence of intestinal extracellular ATP (eATP) also provide superior protection from enteropathogenic infection. Thus, modulation of eATP in the small intestine can affect high-affinity IgA response against gut colonizing bacteria.

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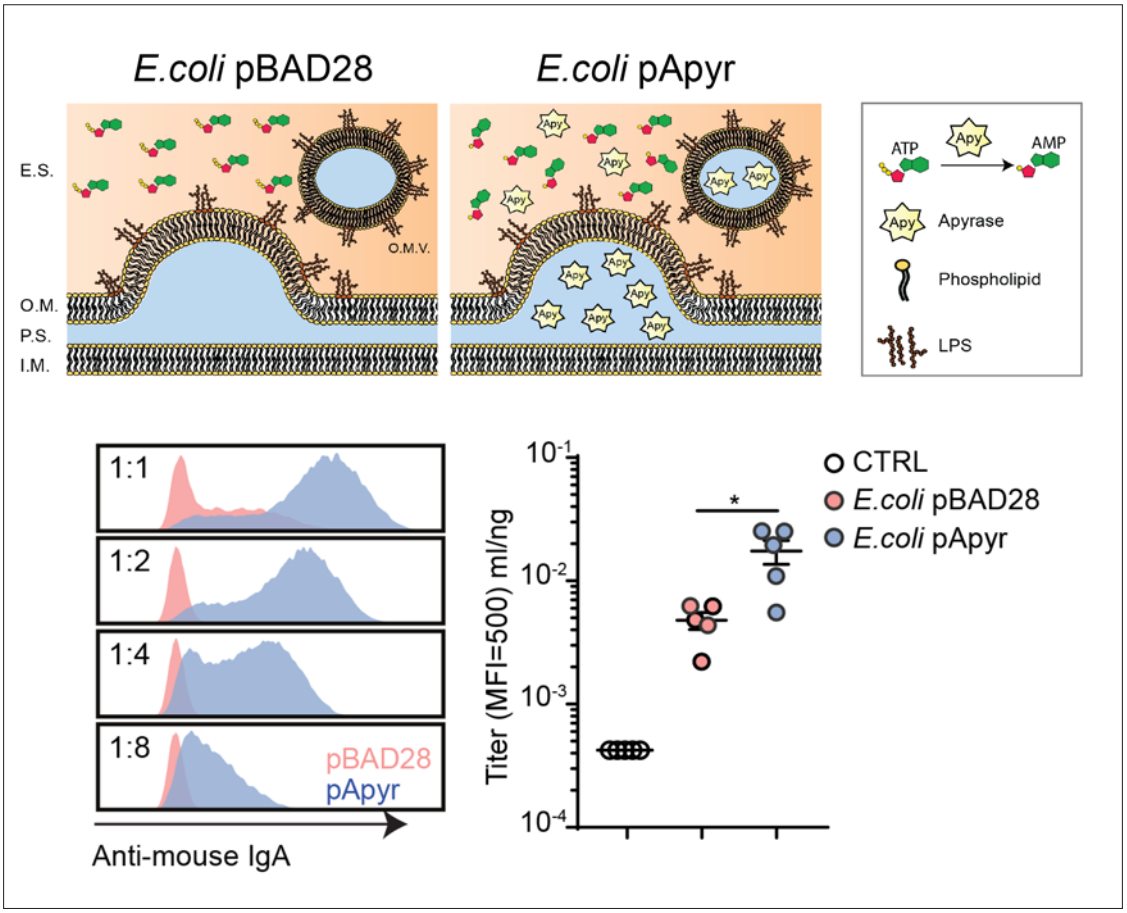

Figure 2.
Enhancement of secretory IgA response by expression of apyrase in live attenuated vaccines.
Schematic model of apyrase release by E. coli transformants (E.S., extracellular space; O.M., outer membrane; P.S., periplasmic space; I.M., inner membrane; O.M.V., outer membrane vesicles). Flow cytometry for anti-E. coli IgA in intestinal wash from mice immunized with E. colip^{BAD28} or E. colip^{Apyr} and intestinal anti-E.coli IgA titer in non-immunized mice (CTRL) and mice immunized with E. colip^{BAD28} or E. colip^{Apyr}.

Senescence induction by the ATP-gated P2X7 receptor limits T cell effector response in the tumor microenvironment

Andrea Romagnani, Elsa Rottoli, Benedetta De Ponte Conti, Elisa Civanelli and Fabio Grassi

Peculiar features of the tumor microenvironment condition the function of infiltrating immune system cells and eventually protect the malignant tissue from eradication. Extracellular adenosine triphosphate (eATP) is a signaling molecule, which variably affects directly or after hydrolysis to adenosine, all cells of the immune system. Whereas eATP is virtually absent in the interstitium of normal tissues, it can be present in the hundreds micromolar range in tumors, a concentration compatible with activation of the ATP-gated ionotropic P2X7 receptor. We show that P2X7 activity in tumor-infiltrating effector T cells limits cell proliferation and tumor suppression. In these cells, deletion of *P2rx7*, encoding for P2X7, promotes a transcriptional signature that correlates with enhanced cytotoxic T cell response in human solid tumors. Conversely, P2X7 stimulation of T effector/memory (TEM) cells leads to generation of mitochondrial reactive oxygen species (ROS) and p38 MAPK dependent upregulation of *cyclin-dependent kinase inhibitor 1A* (*Cdkn1a*, encoding for p21Waf1/Cip1) that result in cell cycle arrest and cellular senescence. Pharmacological antagonism of P2X7 or hypomorphic polymorphism of human P2RX7 prevent Cdkn1a expression and enhance TEM cells proliferation. These results uncover a purinergic checkpoint that limits the effector T cells response within the tumor microenvironment and can be targeted to improve the efficacy of cancer immunotherapy strategies.

P2X7 receptor restrains pathogenic Tfh cell generation in systemic lupus erythematosus

Caterina E. Faliti, Lisa Perruzza, Andrea Romagnani, Benedetta De Ponte Conti and Fabio Grassi

Altered control of T follicular helper (Tfh) cells can lead to generation of autoantibodies and autoimmune manifestations. Signaling pathways that selectively limit pathogenic responses without affecting the protective function of Tfh cells are unknown. We have shown that the ATP-gated ionotropic P2X7 receptor restricts the expansion of aberrant Tfh cells and the generation of self-reactive antibodies in experimental murine lupus, but its activity is dispensable for the expansion of antigen-specific Tfh cells during vaccination. P2X7 stimulation promotes caspase-mediated pyroptosis of Tfh cells and controls the development of pathogenic ICOS$^+$ IFN-γ–secreting cells. Circulating Tfh cells from patients with systemic lupus erythematosus (SLE) but not primary antiphospholipid syndrome (PAPS), a non-lupus systemic autoimmune disease, were hyporesponsive to P2X7 stimulation and resistant to P2X7-mediated inhibition of cytokine-driven expansion. These data point to the P2X7 receptor as a checkpoint regulator of Tfh cells; thus, restoring P2X7 activity in SLE patients could selectively limit the progressive amplification of pathogenic autoantibodies, which deteriorate patients' conditions.

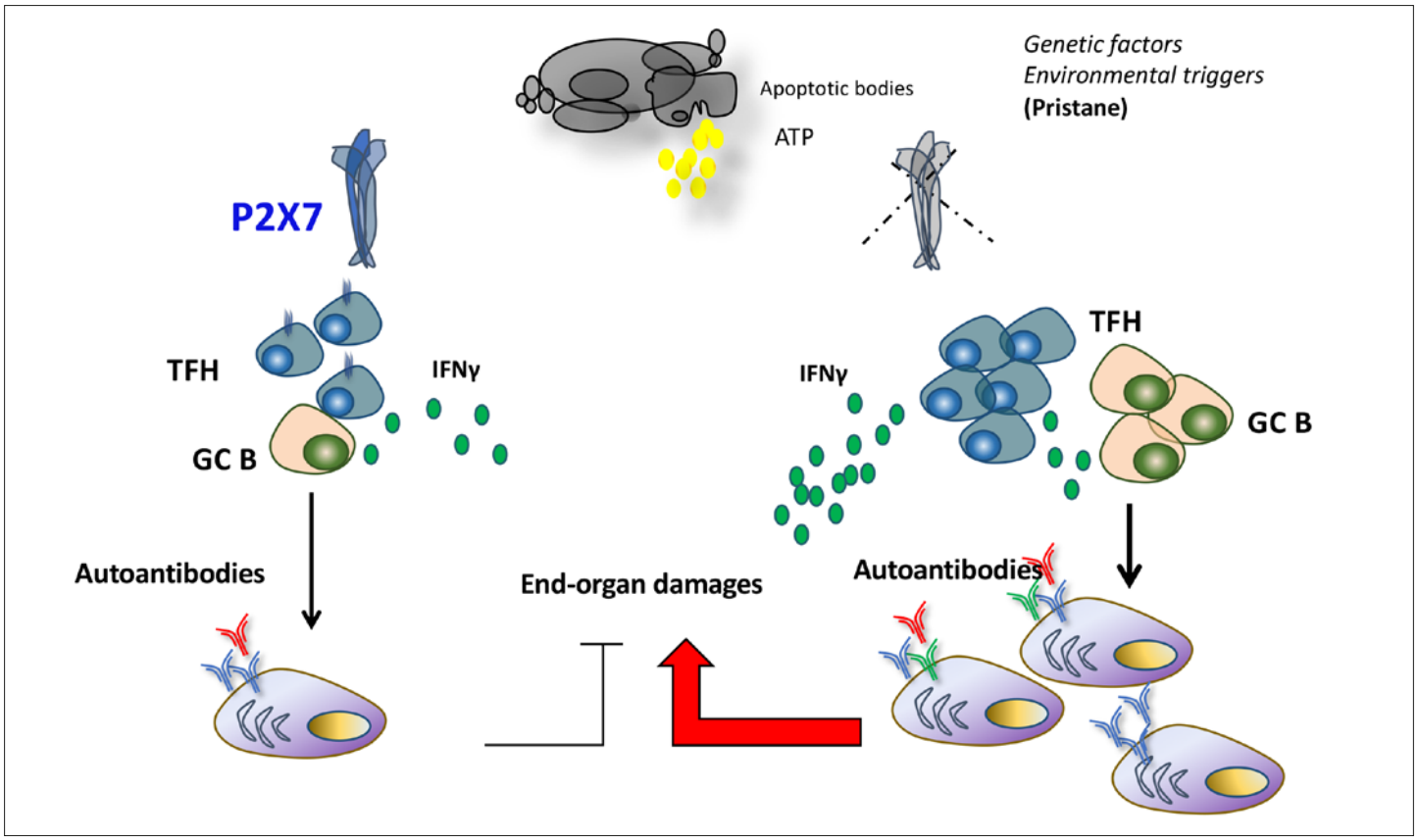

Figure 3.
Deregulated expansion of pathogenic T follicular helper cells by impaired P2X7 activity.
Activation of potentially pathogenic Tfh cells by self-antigens released by apoptotic bodies is limited by P2X7 receptor signaling (left). Lack of P2X7 results in enhanced Tfh cells activity, IFN-γ production and expansion of autoantibody producing plasma cells causing end-organ damage (right).

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Extracellular ATP immunomediated damage in α-sarcoglycan-deficient muscular dystrophy
Fabio Grassi
In muscular dystrophies, muscle membrane fragility results in a tissue-specific increase of danger associated molecular pattern molecules (DAMPs) and infiltration of inflammatory cells. The DAMP extracellular ATP (eATP) released by dying myofibers steadily activates muscle and immune purinergic receptors exerting dual negative effects: a direct damage linked to altered intracellular calcium homeostasis in muscle cells and an indirect toxicity through the triggering of the immune response and inhibition of regulatory T cells. Accordingly, pharmacologic and genetic inhibition of eATP signaling improves the phenotype in models of chronic inflammatory diseases. In α-sarcoglycanopathy, eATP effects may be further amplified because α-sarcoglycan extracellular domain binds eATP and displays an ecto-ATPase activity, thus controlling eATP concentration at the cell surface and attenuating the magnitude and/or the duration of eATP-induced signals. We have shown that *in vivo* blockade of the eATP/P2X purinergic pathway by a broad-spectrum P2X receptor antagonist delayed the progression of the dystrophic phenotype in α-sarcoglycan-null mice. eATP blockade dampened the muscular inflammatory response and enhanced the recruitment of forkhead box protein $P3^+$ immunosuppressive regulatory $CD4^+$ T cells. The improvement of the inflammatory features was associated with increased strength, reduced necrosis, and limited expression of profibrotic factors, suggesting that pharmacologic purinergic antagonism, altering the innate and adaptive immune component in muscle infiltrates, might provide a therapeutic approach to slow disease progression in α-sarcoglycanopathy.

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

Swiss Cancer League
The ATP-gated ionotropic P2x7 receptor as a possible target to enhnce the efficacy of cancer immunotherapy
2017-2019

San Salvatore Foundation
Extracellular ATP as target in tumor environment to improve adaptive immunity
2018-2020

Novartis Foundation
Role of the ATP gated ionotropic P2X7 receptor in regulating T follicular helper cells in systemic lupus erythematosus
2018

Bill & Melinda Gates Foundation
In vivo PK and efficacy studies of Human IgA Monoclonal Antibodies against Enteric Pathogens
2018-2020

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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.

Greta Guarda
Immune Mechanisms

Greta Guarda studied Molecular Biology at the University of Zurich and performed her diploma work at the Swiss Federal Institute of Technology, Zurich. From 2004 to 2007, she carried out her PhD work on T cell-mediated immunity at the Institute for Research in Biomedicine (IRB), Bellinzona. In 2007, she joined as post-doctoral fellow the University of Lausanne, where she became senior lecturer in 2010. During these years, she focused her research on NOD-like receptors and inflammasome function. She established her independent research group in 2012 thanks to the award of a Swiss National Science Foundation professorship and a European Research Council starting grant in 2013. In March 2018, Greta Guarda joined the IRB as Group Leader. For her scientific contributions, she was awarded the "Premio Fondazione Dr. Ettore Balli 2018" and the "Pfizer Research Prize 2019". Since 2016, she is member of the Federal Ethics Committee on Non-Human Biotechnology.

Research Focus

Our research focuses on the interplay between major histocompatibility complex (MHC) class I, cytotoxic T cells, and natural killer (NK) cells in the context of infection and cancer. In fact, recognition of infected or transformed cells by cytotoxic T lymphocytes requires MHC class I molecules. NK cells, using a complementary strategy, eliminate hazardous cellular targets lacking MHC class I expression. These molecules are therefore central players in immunity and we study novel mechanisms – relevant for innate and adaptive cytotoxic responses – regulating their levels. Further, we investigate new molecular pathways controlling function and metabolic fitness of lymphocytes in health and disease. To achieve these goals, we use a variety of approaches, including genetic, genomic, biochemical, and molecular techniques, as well as translational models.

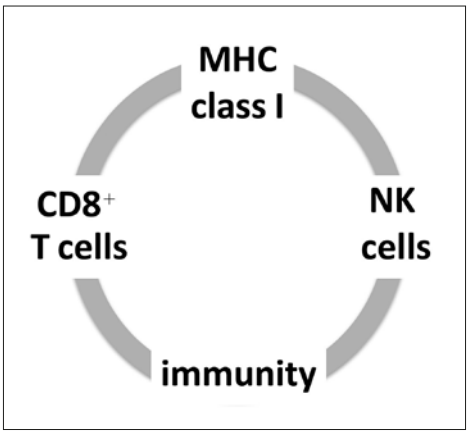

Figure 1.
Global representation of our research interests.

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Members

Sonia Chelbi, PhD – Berenice Fischer, PhD student – Nicolas Fonta, technician – Hanif Javanmard Khameneh, PhD – Pedro Ventura, PhD student – Alessandro Zenobi, technician

Deciphering the role of Nlrc5 in cancer

Berenice Fischer and Greta Guarda

The work of different laboratories, ours included, demonstrated that the NOD-like receptor NLRC5 (NLR, CARD-containing 5) transactivates major histocompatibility complex (MHC) class I genes, thus exerting a transcriptional regulatory function. Given the recent discovery of NLRC5, our understanding of its physiological relevance is still limited, but its function in regulating MHC class I expression suggests NLRC5 to be relevant in cancer immune surveillance. In fact, human data showing that NLRC5 expression correlates with higher patient's survival rates in several cancers are emerging. We are therefore interested in studying the implications of NLRC5 in anticancer T cell-mediated immunity.

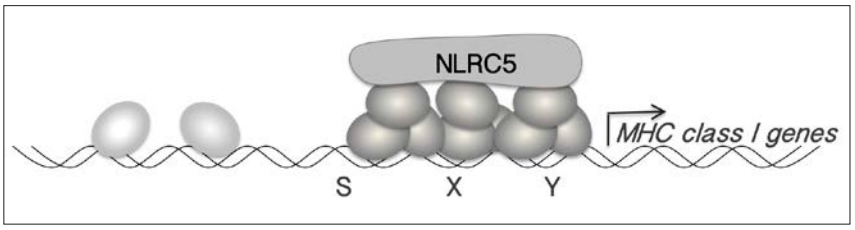

Figure 2.
NLRC5 regulates MHC class I gene transcription.
It occupies the 'SXY module' in the promoter of MHC class I genes by binding to a DNA-binding protein complex known as "enhanceosome".

Understanding the role of Rfx7

Sonia Chelbi, Berenice Fischer, and Greta Guarda

Regulatory factor X 7 (Rfx7), an uncharacterized putative DNA-binding factor, belongs to a family involved in immunity, by regulating major histocompatibility complex (MHC) expression, and in ciliogenesis. Based on phylogenetic analyses and on its high expression levels in hematopoietic cells, we hypothesized that Rfx7 might also control immune cell functions. We found that deletion of Rfx7 led to enhanced activation features in natural killer (NK) cells, affecting NK cell maintenance and immunity. Transcriptomic and genomic approaches showed that Rfx7 coordinated a transcriptional network controlling cell metabolism, an interesting finding with regard to its genetic association with lymphoid malignancies. Through present and future studies, we aim gaining insights into the role of Rfx7 in other lymphocyte subsets.

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Deciphering the role of phosphatases in PD-1 and cytokine signalling

Pedro Ventura, Alessandro Zenobi, Nicolas Fonta, and Greta Guarda

Anticancer T cells acquire a dysfunctional state characterized and maintained by the expression and engagement of inhibitory receptors, such as programmed cell death 1 (PD-1). Although based on in vitro findings, the phosphatase Shp-2 (Src-homology 2 domain-containing phosphatase 2) is considered a key player downstream of these receptors, this remained unexplored in physiological settings. Notably, we found that Shp-2-deficient T cells acquired a dysfunctional state in the tumour microenvironment and responded to PD-1 blockade, indicating the existence of additional signalling factors. Further, Shp-2 has been suggested to regulate signalling downstream of NK cell inhibitory receptors, which are essential to calibrate NK cell responsiveness. However, our genetic approaches did not support this hypothesis. Intriguingly, Shp-2 has been shown to positively regulate ERK (extracellular signal-regulated kinase) activation in various tissues. We thus investigated this possibility in NK cells and identified an essential role for Shp-2 in engaging this cascade downstream of the receptor for interleukin-15, a key cytokine for NK cell development and function. Taken together, these data encourage further investigations of the signalling downstream of inhibitory receptors and of Shp-2's function in cytokine signalling more broadly.

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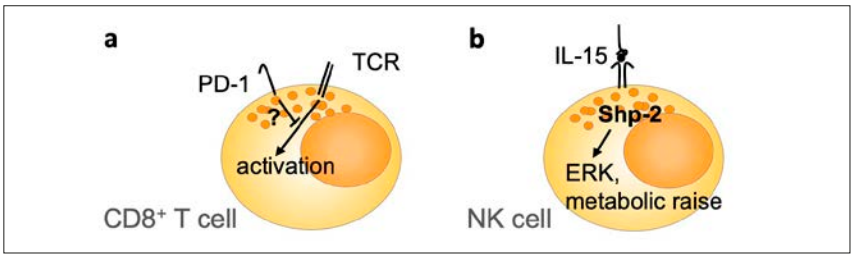

Figure 3.
a. Shp-2 is dispensable for anticancer T cells to acquire a dysfunctional state and downstream of PD-1, indicating the existence of additional inhibitory mechanisms to be discovered.
b. Shp-2 plays an important role to engage ERK and metabolic raise in response to acute IL-15 stimulation.

Deciphering the role of Myc in NK cells

Sonia Chelbi, Nicolas Fonta, Greta

Development, metabolism, and effector functions of natural killer (NK) cells, which are innate cytotoxic lymphocytes important to control infections and cancer, depend on interleukin (IL)-15. This cytokine activates multiple signalling cascades, including the transcription factor signal transducer and activator of transcription 5 (Stat5), the mechanistic target of rapamycin (mTOR), and mitogen-activated protein kinase (MAPK) pathways. Although the protooncogenic transcription factor Myc remains poorly investigated in NK cells, it has been shown to be regulated transcriptionally by IL-15 and stabilized by the mTOR and MAPK cascades. We therefore aim to define the multiple intersection levels of Myc and IL-15 signalling in NK cells, which could help delineate the pathways involved in malignancies originating from these lymphocytes.

Deciphering the role of immunomodulatory bacterial lysates

Hanif Javanmard Khameneh and Greta Guarda

We have an important collaboration with Vifor Pharma, Glattbrugg, Switzerland, aimed at dissecting the immunomodulatory effects of an oral medicine of bacterial origin used for the prevention of respiratory tract infections and now oriented towards airway inflammation disorders such as wheezing, allergic asthma, and asthma exacerbations. Through this project, we aim at detailing the molecular mechanisms underlying the effectiveness of this bacterial extract, gaining insights into general pathways modulating immune responses.

Funding

Swiss National Science Foundation - Professorship
T cell-intrinsic role of pattern recognition receptors
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European Research Council (ERC)
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79280 / 2018-2022

Novartis Foundation
Uncovering the Myc network in NK cells
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Fondazione San Salvatore
Uncovering the T cell-intrinsic role of NLRC5 in antitumoral responses
2018-2020

OM Pharma
Pathways regulation by OM-85
2018-2020

Collaborations

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Publications

Shp-2 is critical for ERK and metabolic engagement downstream of IL-15 receptor in NK cells.
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[1]These authors contributed equally to this work; [2]Shared senior authorship and corresponding author

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Research Groups

Antonio Lanzavecchia
Immune regulation

Lanzavecchia is an immunologist known for his work on antigen presentation, T cell activation, immunological memory and human monoclonal antibodies. 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. 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, 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. In 2017, he received the Robert
Koch Prize and the Sanofi-Institut Pasteur Award and, in 2018 the Louis-Jeantet Prize for Medicine.

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, peptidomics and metabolomics to the study of human T cell differentiation and function.

Team

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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*

In two previously described donors, the extracellular domain of LAIR1, a collagen-binding inhibitory receptor encoded on chromosome 19, was inserted between the V and DJ segments of an antibody. This insertion generated, through somatic mutations, broadly reactive antibodies against RIFINs, a type of variant antigen expressed on the surface of Plasmodium falciparum-infected erythrocytes. 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-endemic regions. Here 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 without conferring enhanced protection against febrile malaria. By analysing the antibody-producing B cell clones at the protein, cDNA and gDNA levels, 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. 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 genomic DNA 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 inserts originating from transcribed genes that, in rare cases, comprised exons with orientations and frames compatible with expression. These results reveal different modalities of LAIR1 insertion that lead to public and dominant antibodies against infected erythrocytes 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, Swiss Tropical Institute, Basel (CH); Peter Crompton, Laboratory of Immunogenetics, NIAID, Rockville, MA (US); Peter Bull, KEMRI-Wellcome Trust Research Programme, Kilifi (KE).

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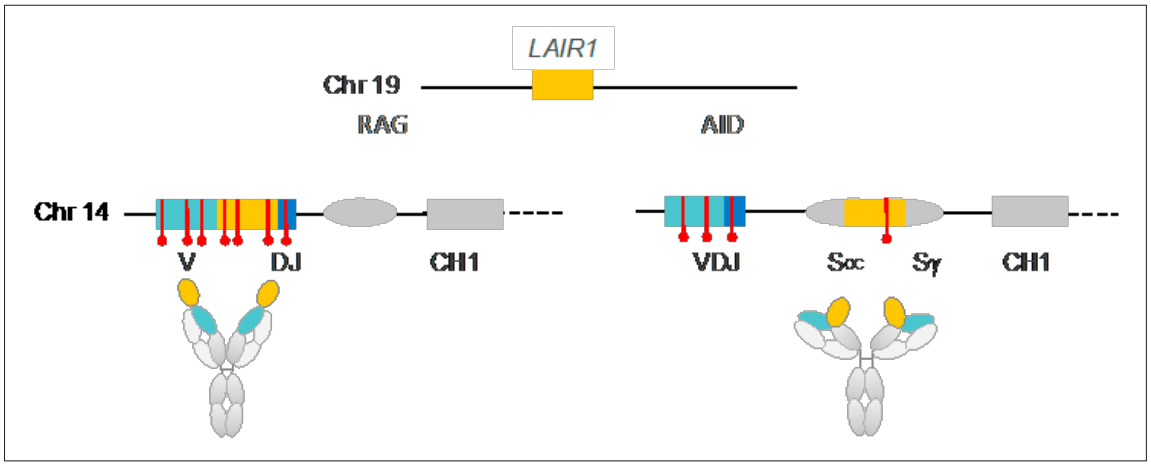

Figure 1.
Templated LAIR1 insertions in the V-DJ junction or in the switch region generate bispecific antibodies with the LAIR1 domain positioned in the CDR3 or in the VH-CH1 elbow, respectively.

A Public lineage of antibodies that potently block malaria infection

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

Immunization with attenuated Plasmodium falciparum sporozoites (PfSPZs) has been shown to protect against malaria, but the features of the antibody response induced by this treatment remain unclear. To investigate this response in detail, we isolated IgM and IgG monoclonal antibodies from Tanzanian volunteers who were immunized with repeated injection of Sanaria PfSPZ Vaccine and who were found to be protected from controlled human malaria infection with infectious homologous PfSPZs. All IgG monoclonal antibodies bound to P. falciparum circumsporozoite protein (PfCSP) and recognized distinct epitopes in its N terminus, NANP-repeat region, and C terminus. Strikingly, the most effective antibodies, as determined in a humanized mouse model, bound not only to the repeat region, but also to a minimal peptide at the PfCSP N-terminal junction that is not in the recombinant RTS,S vaccine. These dual-specific antibodies were isolated from different donors and were encoded by VH3-30 or VH3-33 alleles that encode tryptophan or arginine at position 52. Using structural and mutational data, we describe the elements required for germline recognition and affinity maturation. This study provides potent neutralizing antibodies and relevant information for lineage-targeted vaccine design and immunization strategies.

Collaborators: Claudia Daubenberger, Swiss Tropical Institute, Basel (CH); Brandon Sack, CIDR, Seattle (US) and Ian Wilson, Scripps research Institute, La Jolla, CA.

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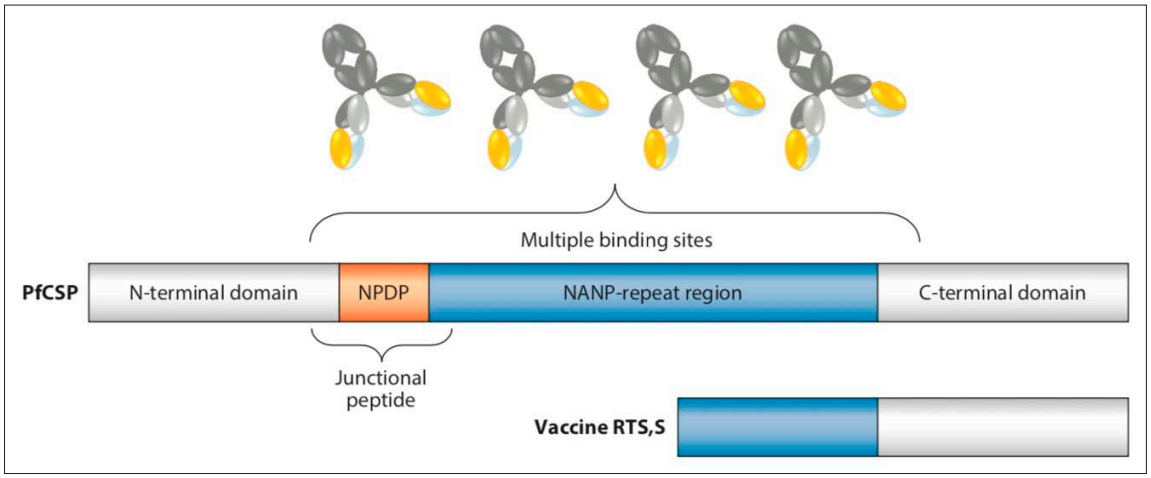
Figure 2.
Potent neutralizing antibodies bind to multiple sites on the CSP protein, including the N-terminal NPDP epitope that is not present in the current RTS,S vaccine.



Human monoclonal antibodies for serotherapy and vaccine design

Luca Piccoli, Laurent Perez, Mathilde Foglierini, Chiara Silacci-Fregni, Blanca Fernandez and Antonio Lanzavecchia

In collaboration with other laboratories we use human monoclonal antibodies isolated from infected individuals to develop new serotherapy approaches and to dissect the mechanisms of protection. We also use the antibodies to design improved vaccines capable of selectively eliciting neutralizing antibodies. A human bispecific monoclonal antibody with high therapeutic potential has been produced against Zika virus. Monoclonal antibodies have been instrumental to elucidate the mechanism of coronavirus fusion and the structure of the influenza hemagglutinin membrane anchor, as well as the mechanism of norovirus evasion. Antibodies to bacterial antigens have been used to visualize bacteria *in vivo* and to demonstrate a new mechanism of bacterial elimination in the intestine through enchained bacterial growth. Monoclonal antibodies have been also instrumental to design stabilized prefusion proteins that work as effective vaccines against four human parainfluenza viruses and against bovine RSV. Finally, antibodies have been instrumental to address fundamental questions on the stability of serological memory and to identify the receptors for the human cytomegalovirus pentameric complex.

Collaborators: Davide Corti, Humabs SA, Bellinzona (CH); Peter Kwong, VRC, NIAID, Bethesda MA (US); George Georgiou, University of Texas, Austin TX (US); David Veesler, University of Washington, Seattle (US); John Skehel, Francis Crick Institute, London (UK); Ralph Baric UNC, Chapel Hill (US); Claudio Ciferri, Genentech, California; Wolf-Dietrich Hardt, ETH Zurich (CH).

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Light chain dimers are unstable and acquire amyloidogenic properties in the absence of selection

Sara Ravasio, Josipa Jerak, Maura Garofalo, Andrea Cavalli and Antonio Lanzavecchia

Immunoglobulin Light (L) chains are an essential component of the B cell receptor (BCR) and contribute to its unique specificity that determines the negative and positive selection of B cell clones. However, L chain homodimers, also known as Bence-Jones proteins, are also secreted by normal and malignant plasma cells and, when present in large amounts, can deposit as amyloid fibrils in target tissues leading to organ failure. Why and how L chains develop these pathogenic properties remains to be established. Here we show that, L chain dimers and are inherently unstable and can be further destabilized by somatic mutations, leading to the exposure of hydrophobic patches and free thiols and to an increased propensity to melt and aggregate into Congo Red-positive, Thioflavin T-positive amyloid fibers under reducing conditions. We also show that L chain dimers have a distinctive specificity and bind to cell surfaces and to the extracellular matrix, a property that may increase their concentration in target tissues. Thus, in the absence of selection for homologous pairing, L chain dimers develop, through somatic mutations, into rogue proteins, leading to concentration-dependent aggregation and organ toxicity.

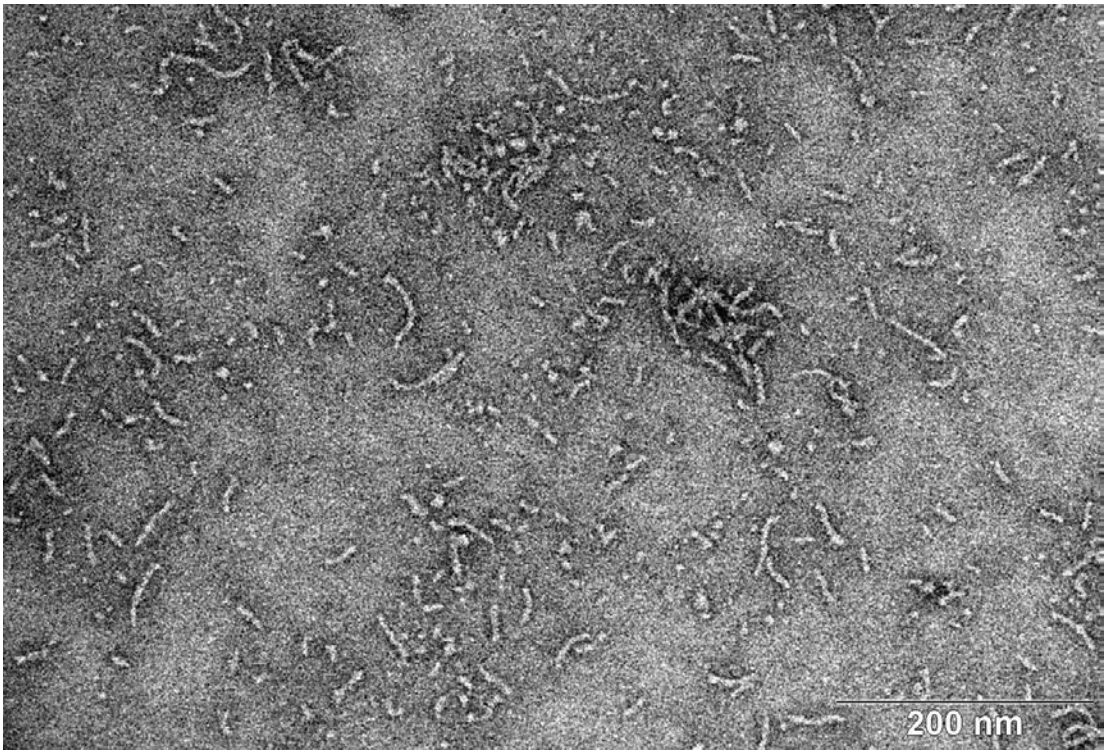

Figure 3.
Light chain dimers aggregate in vitro to form typical amyloid fibrils.

A single T cell epitope drives the neutralizing anti-idiotypic antibody response to natalizumab in patients with multiple sclerosis

Antonino Cassotta, Chiara Silacci-Fregni, Blanca Fernandez Rodriguez, Mathilde Foglierini, David Jarrossay, Federica Sallusto, Antonio Lanzavecchia & Luca Piccoli

Natalizumab (NZM), a humanized monoclonal IgG4 antibody to α4 integrins, is used to treat patients with relapsing-remitting multiple sclerosis (MS), but in about 9% of the cases neutralizing anti-drug antibodies (ADAs) are induced leading to therapy discontinuation. To understand the basis of the ADA response and the mechanism of ADA-mediated neutralization, we performed an in-depth analysis of the B and T cell response in two patients. By characterizing a large panel of NZM-specific monoclonal antibodies, we found that, in both patients, the response was polyclonal and targeted different epitopes of the NZM variable region. The neutralizing activity was acquired through somatic mutations and correlated with a slow dissociation rate, a finding that was supported by structural data. Interestingly, in both patients, the analysis of the CD4+ T cell response, combined with mass spectrometry-based peptidomics, revealed a single immunodominant T cell epitope in the NZM variable region. Collectively, our study identifies the basis of T-B collaboration that drives neutralizing anti-idiotypic antibodies to NZM.Sack, CIDR, Seattle (US) and Ian Wilson, Scripps research Institute, La Jolla, CA.

Funding

Swiss National Science Foundation
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National Institutes of Health (USA)
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Swiss Vaccine Research Institute
Antibody discovery and engineering
2014-2019

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2016-2018

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Publications

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Research Groups

Maurizio Molinari
Protein folding and quality control

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. Between 1998 and 2000, he returned at the ETH-Zurich in the laboratory of Ari Helenius. Since October 2000, he is group leader at the IRB in Bellinzona. The studies performed by Molinari's group at the IRB contributed to the knowledge of mechanisms devised by cells for the production of functional polypeptides and for efficient disposal of folding-defective proteins. Amongst the scientific contributions of Molinari's group, there is i) a novel passive vaccination strategy using an immunoisolated allogeneic implant capable of expressing, processing, and secreting single chain antibodies against the Abeta peptide that was successfully used in a mice model for Alzheimer's disease; ii) the characterization of the intracellular timer based on sequential processing of protein-bound oligosaccharides that decides the time allocated to newly synthesized proteins to complete the folding program before onset of proteasomal degradation; iii) the description of novel autophagy-like mechanisms ensuring recovery from endoplasmic reticulum stress (recovER-phagy) and removal from cells of toxic protein aggregates (ER-to-lysosomes-associated degradation) as hallmarks of several rare diseases caused by intracellular production of faulty gene products. Maurizio Molinari received the Friedrich-Miescher Award 2006. Since 2008, he is Adjunct Professor at the ETH-Lausanne, in 2012 he has been nominated commissary for chemistry and biology teaching at the High Schools in Cantone Ticino and since 2017 gives courses on medicine and scientific research at the Università della Terza Età. He actively participated to the creation of the Rare Diseases Platform recently established in the Italian-speaking part of Switzerland (https://www.malattie-rare-si.ch).

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 selected for degradation. More recently, particular emphasis has been given to the characterization of transcriptional or post translational responses activated by cells experiencing ER stresses, 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, lysosomal storage diseases and Charcot-Marie-Tooth 1B neuropathy. A thorough knowledge of the processes ensuring protein biogenesis and maintenance of cellular proteostasis 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

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Members

Timothy Bergmann, PhD student – Nicole Cesarato, Master student – Elisa Fasana, PhD – Ilaria Fregno, PhD student – Carmela Galli Molinari, MSc – Annamaria Gamper, visiting scientist – Concetta Guerra, PhD student – Marisa Loi, PhD student – Alessandro Marazza, PhD student – Tatiana Soldà, Msc.

Protein Biogenesis in the Mammalian ER

Timothy Jan Bergmann, Carmela Galli Tatiana Soldà and Maurizio Molinari

The endoplasmic reticulum (ER) is a protein factory producing about 40% of the eukaryotic cell's proteome. Nascent polypeptides are welcomed in the ER by public and client-specific molecular chaperones and folding enzymes that ensure attainment of the native structure, which is certified by a quality control machinery that allows secretion of functional proteins. Misfolded proteins are selected for degradation (Fig. 1). We have prepared >50 model folding-competent polypeptides with select physico-chemical features (a selection of which is shown in Fig. 2A), whose fate will be monitored in mammalian cultured cells. How the polypeptide's features determine engagement of specific folding and quality control pathways will be determined in molecular details in wild type cells and in cells ablated of select folding and quality control pathways (on genetic pathway ablation or on pharmacologic pathway activation/inactivation). The results of these studies will inform on general mechanisms ensuring protein biogenesis and quality control in the mammalian cell.

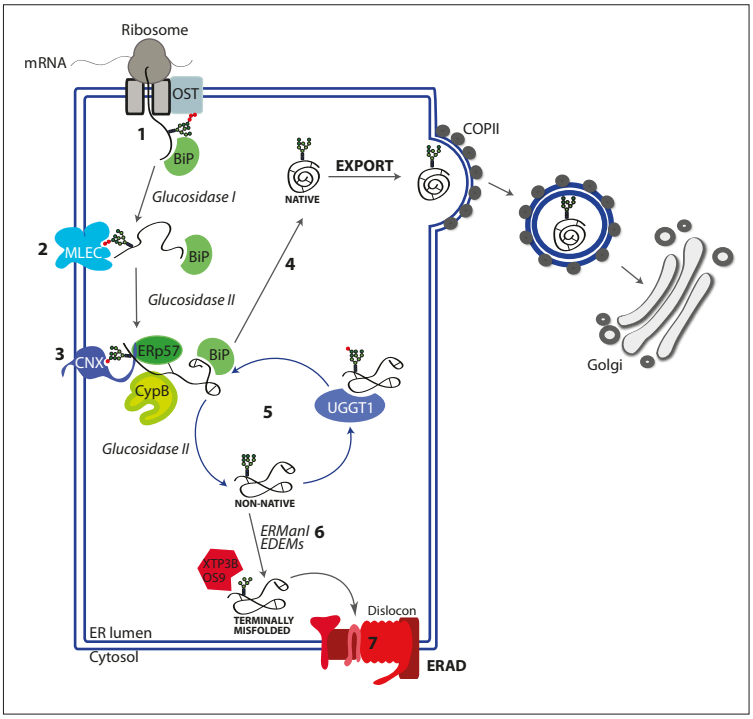

Figure 1.
Membrane-bound ribosomes inject nascent chains in the ER lumen. The luminal chaperone BiP and addition of pre-assembled oligosaccharides by oligosaccharyltransferase (OST) on asparagines in Asn-Xxx-Ser/Thr (N-X-S/T) consensus sequences facilitate translocation and prevent aggregation of unfolded chains (1). Glucosidase I generates di-glucosylated N-glycans that engage MLEC (2). Glucosidase II produces mono-glucosylated glycans that engage CNX, CRT and associated oxidoreductase ERp57 and peptidyl-prolyl isomerase CypB (3). Removal of the last glucose by glucosidase II releases native proteins from CNX/CRT and promotes their export (4). Non-native proteins are re-glucosylated by UGGT1 for prolonged retention in the folding environment (5), which is eventually interrupted on de-mannosylation by ERManI and EDEM proteins (6). Mannose-binding lectins OS9 and XTP3B facilitate retro-translocation of terminally misfolded polypeptides into the cytosol for proteasome-mediated degradation (7). Red circles are terminal glucose residues.

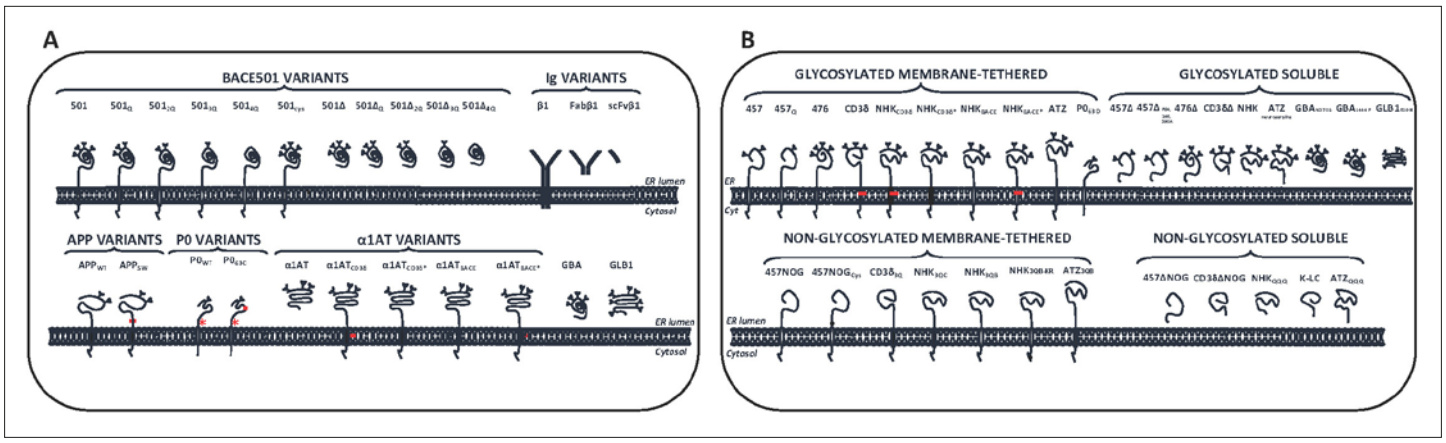

Figure 2.
A Partial selection of available folding-competent polypeptides. B Partial selection of folding-defective polypeptides.

Comparative Interactomics to Identify Novel ER-Resident Quality Control Players

Concetta Guerra 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 (UNIL). 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.

Clearance from the ER of Disease-Causing Faulty Gene Products: the Cytosolic Connection

Timothy Jan Bergmann, Elisa Fasana, Ilaria Fregno, Carmela Galli, Concetta Guerra, Alessandro Marazza, Tatiana Soldà and Maurizio Molinari

Volpi et al
PLoS Genetics. 2019;
15:e1008069

Protein folding is error-prone and misfolding rates are further enhanced on sporadic or hereditary mutations in the polypeptide sequence that are frequently linked to debilitating diseases (protein misfolding disorders). The vast majority of misfolded polypeptides produced in the ER are dislocated across the ER membrane to be degraded by cytosolic 26S-proteasomes in processes collectively defined as ER-associated degradation (ERAD, Fig. 3A). Dislocation across the ER membrane is regulated by multimeric complexes built around one of the several (>20) membrane-embedded E3 ubiquitin ligases expressed in the mammalian ER. As for entering folding programs, also the engagement of select degradation pathways depends on 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, propensity to form aggregates). To better understand the rules governing protein clearance from the ER, we are investigating the fate of >50 model faulty gene products (a selection of which is shown in Fig. 2B) causing gain-of-toxic-function disorders linked to protein aggregation (e.g., α1-antitrypsin deficiency or neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's) or loss-of-function disorders such as lysosomal storage diseases (GM-1, Gaucher, Krabbe and various types of mucopolysaccharidoses including the Hunter's syndrome).

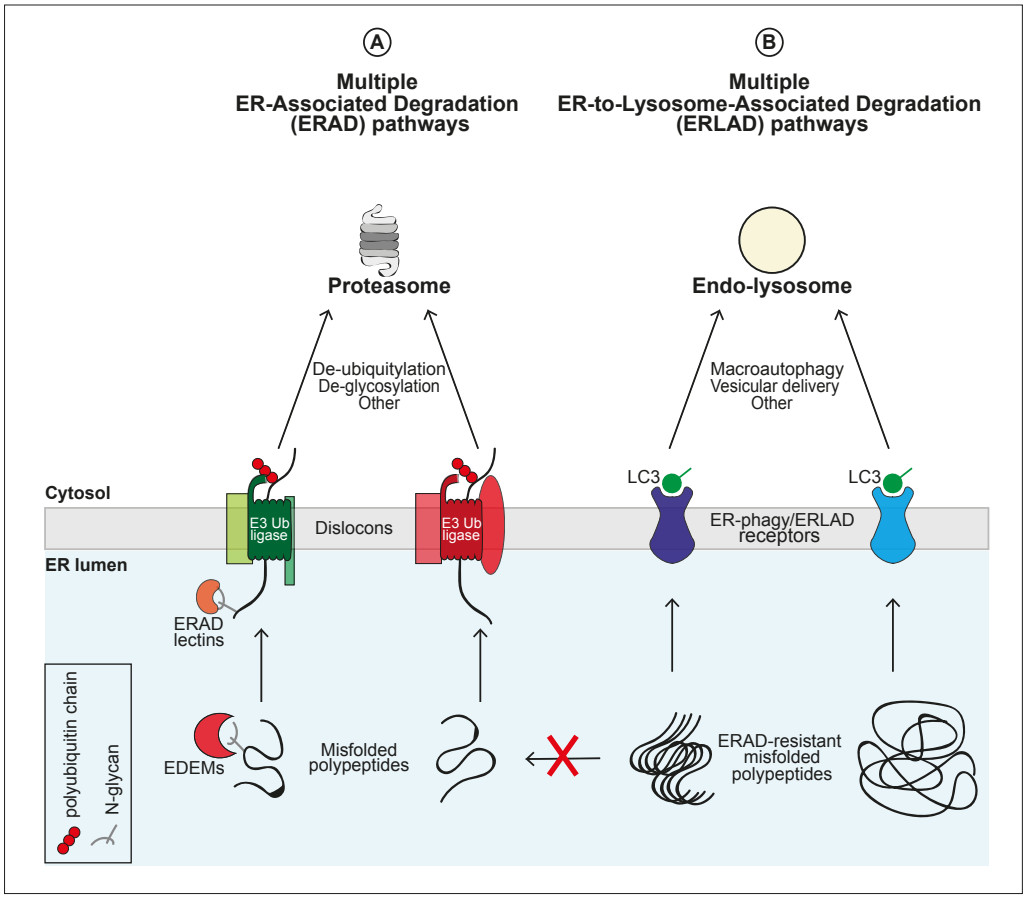

Figure 3.
Degradation pathways for gene products destined to the ER. The figure illustrates the multiple pathways ensuring removal of ERAD clients form the ER lumen (A) or of proteins that cannot enter the ERAD pathways and are cleared from the ER by ERLAD (B).

Role of Membrane-Bound Oxidoreductases in Protein Biogenesis and Disposal

Concetta Guerra and Maurizio Molinari

Guerra et al
Biochem Biophys Res Commun. 2018; 503:938-943.

The lumen of the ER contains 23 PDI members that insure formation of the correct set of intra- and inter-molecular disulfide bonds as a crucial, rate-limiting reaction of the protein folding process. Likewise, some PDI members regulate reduction of intra- and inter-molecular disulfides as an essential reaction promoting dislocation across the ER membrane of misfolded polypeptides to be delivered into the cytosol for proteasomal degradation. 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). We aim at understand the role in protein biogenesis of the 5 type I membrane-bound members of the PDI family (TMX1, TMX2, TMX3, TMX4 and TMX5, Fig. 4). 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 (collaboration with Manfredo Quadroni, UNIL). 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. Recently, we reported that TMX1 preferentially acts on membrane-bound misfolded polypeptides to promote their proteasomal clearance from the ER lumen.

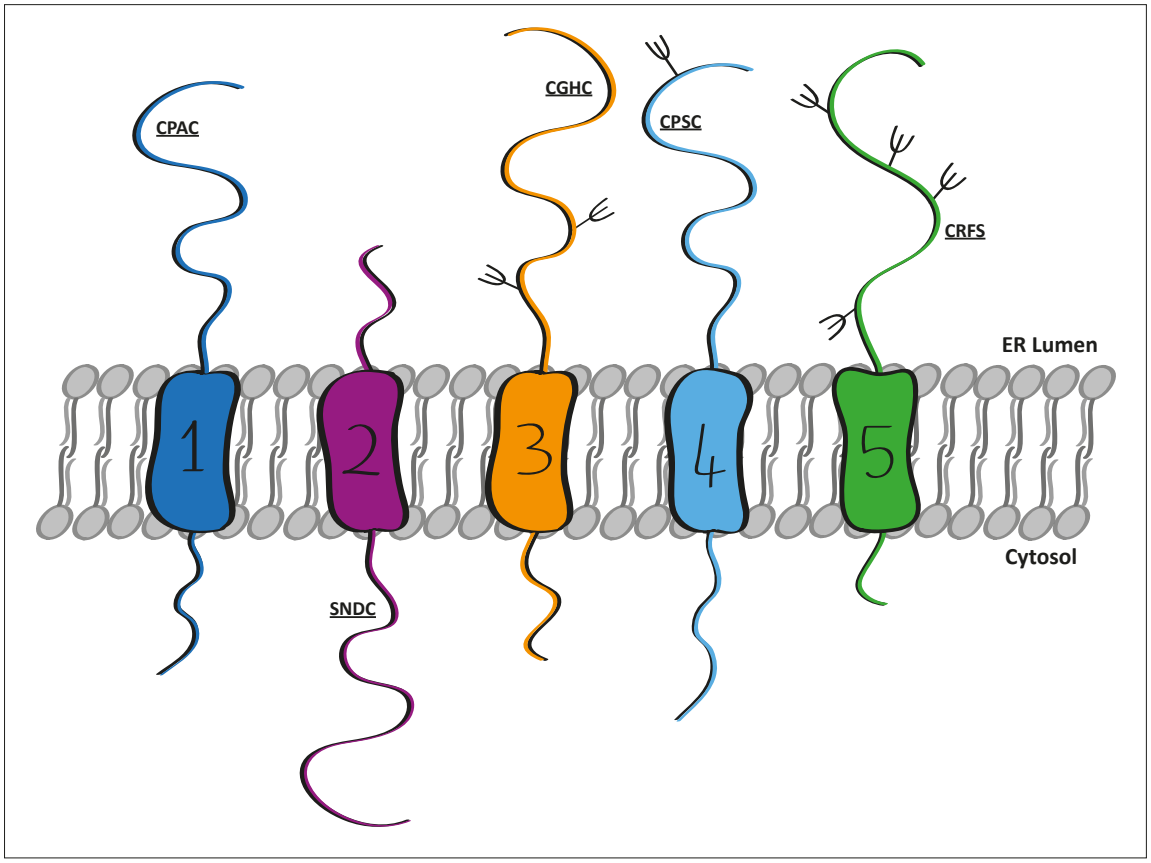

Figure 4.
Structure of the TMX members of the PDI superfamily. The sequence of the catalytic sites is given, and the presence of N-glycans is highlighted in TMX3, TMX4 and TMX5.

Substrate-Specific Mechanisms of Protein Degradation from the ER: the Cytosolic Connection

Timothy Jan Bergmann, Elisa Fasana, Ilaria Fregno, Carmela Galli, Concetta Guerra, Alessandro Marazza, Tatiana Soldà and Maurizio Molinari

Most proteins that fail to attain the native structure in the ER are dislocated into the cytosol for ER-associated degradation operated by 26S proteasomes. However, an increasing number of proteins has been found to be resistant to ERAD. The large size (as in the case of mutant collagen) or the propensity to form aggregates or polymers in the ER lumen (as for mutant serine protease inhibitors (serpins)) may hamper the dislocation across the ER membrane. These faulty gene products must be delivered to the lysosomal compartments for degradation, with mechanisms that are poorly defined. To establish the molecular details of lysosome-mediated clearance of faulty gene products from the ER (we named this catabolic pathway ER-to-Lysosome-Associated Degradation, ERLAD), we take advantage of the large number of model polypeptides available in our lab including collagen variants or ATZ (Fig. 2B), a polymerogenic, mutant member of the serpin superfamily linked to the most common form of alpha1-antitrypsin deficiency. In the case of ATZ, we recently reported on a novel catabolic pathway controlled by several autophagy genes (LC3, ATG4B, ATG5 and ATG7) operating in non-autophagic pathways to ensure segregation of misfolded polypeptides in ER subdomains that vesiculate and eventually fuse with endolysosomes on intervention of the ER-resident SNARE protein syntaxin-17 and the lysosomal SNARE VAMP8 (Fig. 5). In the case of misfolded collagen, the same autophagy genes intervene in a more conventional macro-autophagy pathway to clear from cells ER subdomains containing the aberrant polypeptides. These data reveal client-specific pathways (Fig. 3B). Availability of a vast array of ERAD-resistant faulty gene products will led us to characterize the variety of catabolic pathways activated by mammalian cells to efficiently remove faulty gene products and maintain cellular proteostasis (i.e., the capacity to produce the cellular proteome in appropriate quality and quantity). charidoses including the Hunter's syndrome).

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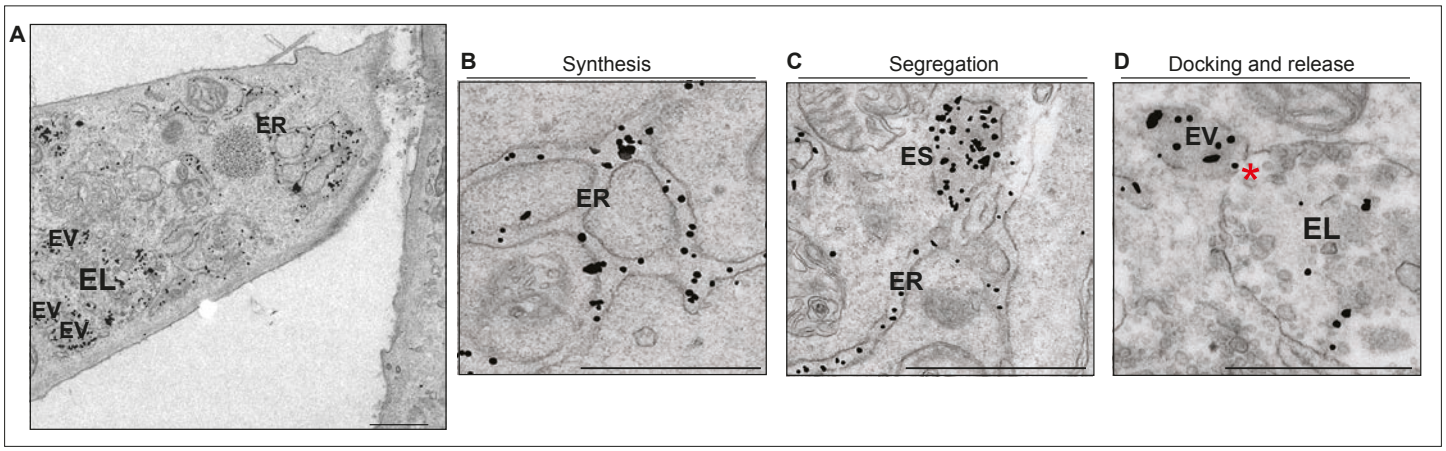

Figure 5.
A Intracellular distribution of gold-labeled ATZ by immunoelectron microscopy. B-D Gold-labeled ATZ in the ER, ER subdomains (ES) and within endolysosomes (EL). The red asterisk shows an ER-derived vesicle (EV) releasing ATZ within an endolysosome.

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Cellular Responses to Variations in ER Homeostasis and Protein Load

Timothy Jan Bergmann, Concetta Guerra, Carmela Galli and Maurizio Molinari

The equilibrium between protein synthesis, export and ERAD is crucial to maintain 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 ER-stresses. Such stresses activate unfolded protein responses (UPR), i.e., adaptive, transcriptional and translational programs that induce the expression of ER resident gene products, increase the ER size and reduce synthesis of cargo proteins 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 (Fig. 6) 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 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 Andrea Rinaldi, IOR, Bellinzona) with proteome analysis, label-free quantitative (LFQ) 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 analysed 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.

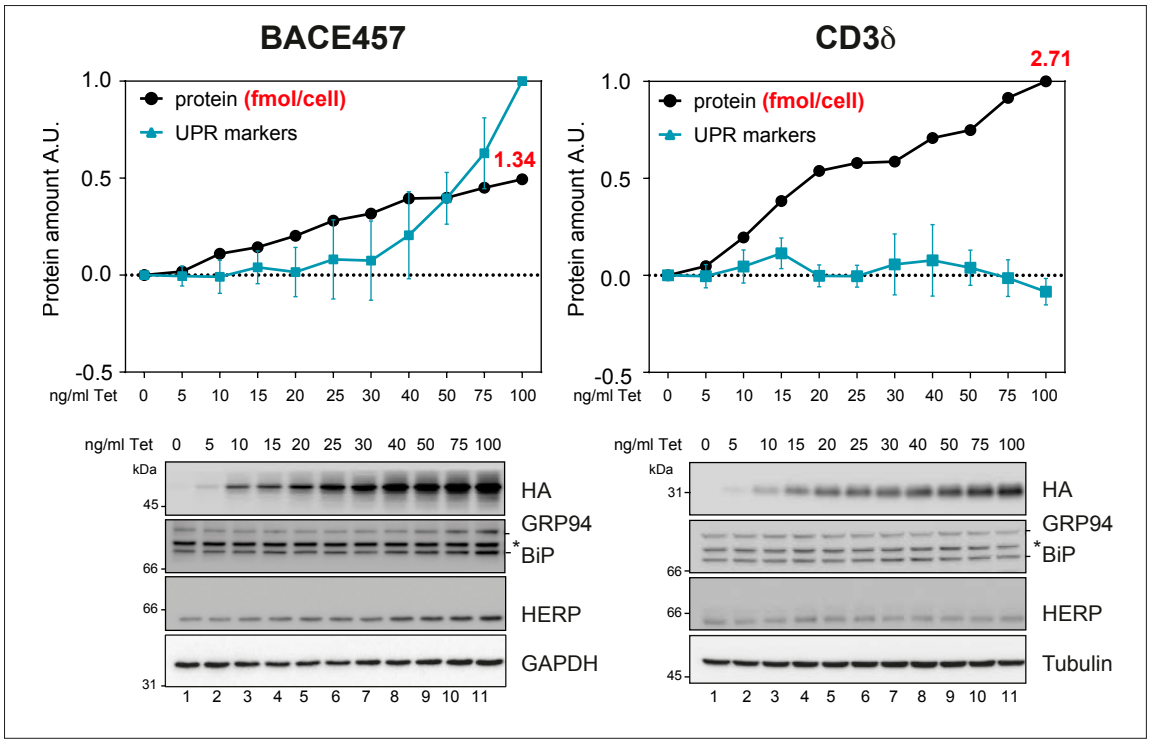

Figure 6.
Inducible protein expression and cellular responses. Tet-induced expression of BACE457 (upper panel, left) and CD3δ (upper panel, right). BiP, GRP94 (middle panels) and HERP (lower panels) levels. Last lane, Tun-induced ER stress (5μg/ml). The graphs show variations of BACE457 (grey, left) 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.

Misfoldable HaloTag® to investigate cellular responses to expression of aberrant gene products

Concetta Guerra, Carmela Galli and Maurizio Molinari

HaloTag® is a Phe272His modified version of the bacterial enzyme haloalkane dehalogenase engineered to covalently and irreversibly bind synthetic chloroalkane reactive ligands for different applications from protein purification to imaging. Several HaloTag versions have been developed by Promega over the years. HaloTag2 can be conditionally misfolded on binding of hydrophobic ligands. We prepared a series of model chimeric proteins by modification of an ER-resident GFP-HaloTag2 chimera (ER-HT, Fig. 7) used by Crews et al. to investigate ER stresses triggered on ligand-induced HaloTag2 misfolding in the ER lumen. Relying on studies showing that transmembrane domains of about 17 residues retain membrane proteins in the ER and their elongation by 5 residues promotes secretion, we have modified the ER-HT construct by introducing two different membrane anchors of 17 (TM17) or 22 residues (TM22). Variants lacking the N-glycan (TM17Q and TM22Q) and displaying the misfoldable unit in the cytosol were also prepared (Fig. 7). The model proteins shown in Fig 7, whose fate will be monitored in the absence or presence of the destabilizing ligand HyT36, will be instrumental i) to screen compounds that modulate ER or cytosolic quality control pathways regulating secretion of native (in the absence of HyT36) or of folding-defective polypeptides (in the presence of HyT36) or secretion pathways that regulate intracellular traffic of membrane proteins; ii) to perform transcriptome and proteome analyses) to characterize cell responses to HyT36-induced misfolding of the various chimeric proteins. We are particularly interested in determining differences between cellular responses to i) misfolding in the ER of the soluble vs. the membrane-tethered HaloTag2 domain; ii) misfolding of the glycosylated vs. the non-glycosylated HaloTag2 domain; iii) misfolding of the HaloTag2 domain in the ER vs. misfolding induced in post ER compartments; iv) misfolding of the HaloTag2 domain in the ER lumen vs. misfolding of the same domain in the cytosol; v) finally, we will investigate the fate of the ER, Golgi, or plasma membrane-localized constructs on HyT36-induced misfolding (e.g., to elucidate the mechanisms of disposal from the various cellular compartments on misfolding-induction). To analyze transcriptional and post-translational responses to perturbed ER homeostasis and to variations in cargo load we have established collaborations with Paola Picotti (ETH-ZH) and Manfredo Quadroni (UNIL) for proteomic and with Andrea Rinaldi (IOR, Bellinzona) for transcriptomic analyses.

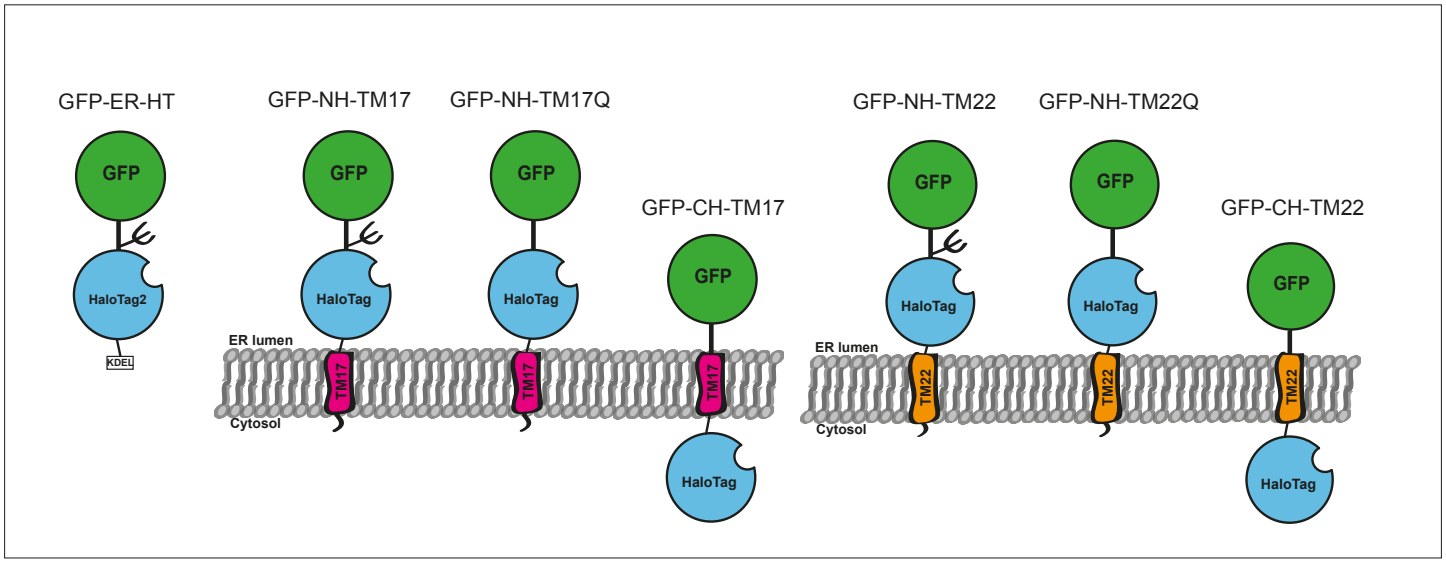

Figure 7.
The soluble ER-HT and the 6 chimeras generated in our lab. NH, the misfoldable HaloTag2 domain is at the N-terminus of the membrane anchor (i.e., luminally oriented); CH at the C-terminus (i.e., cytosolic). TM17/22 shows the length of the hydrophobic, transmembrane domain; Q shows the Asn to Gln sequon mutation that prevents addition of the oligosaccharide. Intracellular trafficking is monitored in CLSM ($GFP_{488Ex/509Em}$).

Revealing Mechanisms Regulating Recovery from Transient ER Stress in Mammalian Cells

Marisa Loi, Carmela Galli, Tatiana Soldà and Maurizio Molinari

Eukaryotic cells respond to perturbation of 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). If this happens, recovery programs are activated that re-establish pre-stress ER size, chaperone content and compartmental function.

We have recently established experimental conditions eliciting acute ER stress in mammalian cells on challenging mammalian cells with cyclopiazonic acid (CPA), a reversible inhibitor of the calcium pump embedded in the ER membrane. On CPA washout, we observed a rapid reduction of the ER stress-induced gene's transcripts. Subsequently, the size of the ER and the level of the ER stress-induced chaperOME return to pre-stress levels on activation of the "ER-phagy receptor" function of the translocon component SEC62. This engages cytosolic components of the autophagic machinery to deliver excess ER subdomains to RAB7/LAMP1-positive endolysosomes for clearance in processes that re-establish physiologic ER homeostasis collectively defined as recovER-phagy. Together with FAM134B, RTN3 and ATL3 (activated on nutrient deprivation) and CCPG1 (activated on ER stress), SEC62 is one of the five ER-resident, LC3-binding "ER-phagy receptors" involved so far in selective turnover of the mammalian ER (Fumagalli et al Nature Cell Biol 2016). The engagement of autophagy gene products by ER-phagy receptors suggests the involvement of catabolic pathways relying on macro-ER-phagy, where ER subdomains are captured by double-membrane autophagosomes in their way to lysosomal clearance. Studies are ongoing to characterize recovER-phagy in molecular details.

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Funding

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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

Fondazione Comel
Studies on conformational proteopathies

Alpha 1 Foundation
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2017-2019

Mizutami Foundation
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2018

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Research Groups

Silvia Monticelli
Molecular Immunology

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 post-transcriptional mechanisms of regulation of gene expression in the activation and function of cells of the immune system.

Research Focus
Our lab is interested in understanding mechanisms of regulation of gene expression in cells of the immune system, including transcription factors, microRNAs (miRNAs) and epigenetic modifications. Epigenetic inheritance is 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 miRNAs. Our lab is 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, miRNA expression and transcription factor-binding. 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. In our lab we are studying the role of DNA methylation, transcription factors 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 applications leading to disease intervention.

Team

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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. 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 catalysing this process are implicated in the pathogenesis of a variety of diseases and in regulating the function of immune cells.

We found that 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 an altered methylation pattern show abnormal proliferation and respond with exaggerated responses to normal stimuli, leading to unrestrained inflammation.

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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. Epigenetic modifications such as DNA methylation are crucial to the appropriate regulation of gene expression in T lymphocytes, ultimately influencing all aspects of T cell biology. We determined the dynamics of modifications of cytosines in the genomic DNA (5mC and 5hmC), and addressed whether DNA demethylation processes linked to T cell activation occur primarily through active or passive processes. We found that activation of primary human naïve and memory T lymphocytes led to a global reduction of genomic 5mC and 5hmC, which was crucial for the proper acquisition of T cell effector functions. However, while all T cells relied primarily on proliferation-dependent processes to dilute DNA modifications, naïve T cells appeared to also take advantage of an active component of 5hmC removal.

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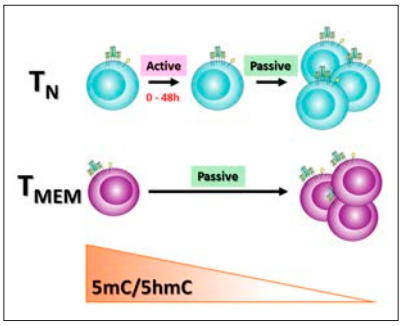

Figure 1.
Activation of primary human naïve and memory T lymphocytes leads to a global reduction of genomic 5mC and 5hmC. While all T cells rely primarily on proliferation-dependent processes to dilute DNA modifications, naïve T cells appear to also take advantage of an active component of 5hmC removal, at least in a window of time following activation and preceding the initiation of proliferation. TN: Naïve T lymphocytes; TMEM: memory T lymphocytes

Role of transcription factors in regulating human T cell activation and functions
Stefan Emming, Niccolò Bianchi 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 are studying the biological relevance of such candidates in human T lymphocytes.

MicroRNAs in the regulation of human T lymphocytes
Michele Chirichella, Stefan Emming, Niccolò Bianchi 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 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 lymphocyte functions at homeostasis and in disease.

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Cancer Letters. 2018; 430:172-178

Funding

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31003A_175569 / 2018-2022

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2017-2020

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Post-doctoral Fellowship to Sara Montagner / 2016-2017

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2016-2017

Ceresio Foundation
Fellowship contribution towards the salary of one PhD student / 2017-2018

Swiss Multiple Sclerosis Society
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Vontobel Stiftung
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Monticelli, S. and G. Natoli
Nat Rev Immunol. 2017; 17:595-607.

Research Groups

Federica Sallusto
Cellular immunology

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. Since 2017, she is Full Professor in Medical Immunology at the ETH Zurich and at the Università della Svizzera italiana (USI), Lugano (joint professorship). 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, of skin-homing Th22 cells and of two distinct types of Th17 cells with pro-inflammatory and regulatory properties. Her recent work demonstrated that the T cell response induced by pathogens or vaccines comprise not only clones polarized toward a single fate, but also clones whose progeny have acquired multiple fates, providing evidence of intraclonal functional heterogeneity in vivo. She also developed a high-throughput cellular screening method based on libraries of polyclonally expanded T cells and used this method to show that human T cells specific for Mycobacterium tuberculosis are largely contained in a non-conventional Th1 subset and to dissect the T cell response in Dengue and Zika infected individuals. The T cell library method was also used 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 and to identify autoreactive T cells in patients with narcolepsy. 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 Science 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 and is currently President elect of the European Federation of Immunological Societies (EFIS). In 2018, she became member of the National Research Council of the Swiss National Science Foundation (SNSF).

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 are expected to provide relevant information for the design of new and more effective vaccine strategies. We are also characterizing human T cells that are induced by commensal microbes to define their functional properties and pattern of reactivity in the steady state and in inflammatory conditions. By applying the same experimental approaches, we conduct 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 and how not harmful environmental antigens or self-antigens can cause pathology (allergy and autoimmunity) in some individuals. Recently, we have identified conditions for efficient gene knock in human T cells using the CRISPR/Cas9 system. The system has great potential not only to define physiological mechanisms of T cell activation, differentiation and plasticity, but also to find ways to engineer more effective T cells for immunotherapy.

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An immunoregulatory and tissue-residency program modulated by c-MAF in human Th17 cells

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

Aschenbrenner D et al.
Nat Immunol. 2018, 19:1126-1136.

Different types of effector and memory T lymphocytes are induced and maintained in protective or pathological immune responses. In this study, we characterized two human CD4$^+$ Th17 cell subsets that, in the recently activated state, could be distinguished on the basis of their expression of the anti-inflammatory cytokine IL-10. IL-10$^+$ Th17 cells upregulated a variety of genes encoding immunoregulatory molecules, as well as genes whose expression is characteristic of tissue-resident T cells. In contrast, IL-10$^-$ Th17 cells maintained a pro-inflammatory gene-expression profile and upregulated the expression of homing receptors that guide recirculation from tissues to blood. Expression of the transcription factor c-MAF was selectively upregulated in IL-10$^+$ Th17 cells, and it was bound to a large set of enhancer-like regions and modulated the immunoregulatory and tissue-residency program. Our results identify c-MAF as a relevant factor that drives two highly divergent post-activation fates of human Th17 cells and provide a framework with which to investigate the role of these cells in physiology and immunopathology.

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

Transcriptional signature of human pro-inflammatory Th17 cells identifies reduced IL-10 gene expression in multiple sclerosis.

Samuele Notarbartolo, Dominik Aschenbrenner, and Federica Sallusto

Hu D, Notarbartolo S, et al.
Nat Comm. 2017, 8:1600.

Our collaborators have previously reported the molecular signature of murine pathogenic Th17 cells that induce experimental autoimmune encephalomyelitis (EAE). In this study. we show that human peripheral blood IFN-γ^+IL-17$^+$ (Th1/17) and IFN-γ–IL-17$^+$ (Th17) CD4$^+$ T cells display distinct transcriptional profiles in high-throughput transcription analyses. Compared to human Th17 cells, human Th1/17 cells have gene signatures with marked similarity to mouse pathogenic Th17 cells. Assessing 15 representative signature genes in patients with multiple sclerosis, we find that Th1/17 cells have elevated expression of *CXCR3* and reduced expression of *IFNG*, *CCL3*, *CLL4*, *GZMB*, and *IL10* compared to healthy controls. Moreover, higher expression of *IL10* in Th17 cells is found in clinically stable vs. active patients. Our results define the molecular signature of human pro-inflammatory Th17 cells, which can be used to both identify pathogenic Th17 cells and to measure the effect of treatment on Th17 cells in human autoimmune diseases.

Collaborators: Vijay K. Kuchroo and Howard L. Weyner, Harvard Medical School, Boston (US), Marco Gattorno, G. Gaslini Scientific Institute, Genova (IT).

Clonotypic and functional heterogeneity of early primed CD4+ naïve T cells

Daniela Vaqueirinho, Mengyun Hu, and Federica Sallusto

Priming of CD4$^+$ T cells can lead to the generation of distinct types of effector and memory T cells that differ in their functional and migratory properties and, thus, in their capacity to eliminate distinct pathogens in specific tissues. Frequently, the CD4$^+$ T cell response to a certain pathogen is dominated by a specific subset that is most adequate to respond to it. However, our laboratory has shown that memory CD4$^+$ T cells specific to certain pathogens can have a high functional heterogeneity and individual clonotypes within this population are present in distinct functional subsets, presumably through a process of intraclonal diversification. We also shown that a single naïve T cell is able to generate multiple effector fates even after a single round of in vitro priming. To further understand the mechanisms of generation of multiple CD4$^+$ T cell fates, we set out to assess the heterogeneity and composition of populations of primed naïve CD4+ T cells at earlier time points after activation. To this end, we have proceeded to the optimization of the detection and isolation of CFSElo pathogen specific CD4$^+$ T cells at day 4-5 after priming with antigens from *Candida albicans* or Influenza viruses. Thereafter, each of the distinct CFSElo populations present at this time point was sorted and, through T cell cloning and re-stimulation, analysed for specificity. In future work, these pathogen-specific early primed CD4$^+$ T cell populations will be assessed for their clonotypic and functional heterogeneity, through techniques such as TCR sequencing and single-cell RNA sequencing. This will allow a better elucidation of the early events that occur after priming of naïve CD4$^+$ T cells and, specifically, to unravel the mechanisms which allow the progeny of a naïve T cell to acquire multiple fates.

Antigen dependent TCR repertoire relation of human cT_{FH} and non-cT_{FH} cells

Mengyun Hu, Antonino Cassotta, Antonio Lanzavecchia and Federica Sallusto

Human cT_{FH} (circulating follicular helper T) cells are a subset of $CD4^+$ memory T cells in peripheral blood that express the chemokine receptor CXCR5. Our understandings about the relationship between cT_{FH} cells and non-cT_{FH} cells, as well as between cT_{FH} and bona fide T_{FH} cells that develop in the germinal centers (GC-T_{FH} cells) in humans is incomplete. We are combining analysis of the antigen-specificity of cT_{FH} and non-T_{FH} cells with TCR repertoire analysis. We found that C. albicans- or tetanus toxoid (TT)-specific cT_{FH} cells are clonally related to their non-cT_{FH} counterpart, while influenza virus-specific cT_{FH} and non-cT_{FH} cells are clonally distinct. Ex-vivo stimulation demonstrated that C. albicans and TT-specific cells were enriched in the ICOS–PD1– cT_{FH} subset, while Flu-specific cells were enriched in the $PD1^+$ cT_{FH} subset. Future plan is to further discriminate between ICOS–PD1– and $PD1^+$ subpopulations of cT_{FH} cells with respect to their clonal relations with non-cT_{FH} cells, and their phenotypic resemblance with GC-T_{FH} cells.

T helper cell response in patients with inborn errors of immunity to Mycobacterium and/or Candida species

Federico Mele, Daniela Latorre, Sandra Jovic, and Federica Sallusto

Most known primary immunodeficiencies (PIDs) are associated with infections caused by multiple microbes. Mendelian Susceptibility to Mycobacterial Diseases (MSMD) is a PID characterized by severe infections due to weakly virulent mycobacteria; MSMD-causing mutations have been identified in ten genes involved in the IL-12/IL-23-IFN-γ immune axis. Isolated inherited chronic mucocutaneous candidiasis disease (CMCD) is characterized by severe, chronic, mucocutaneous infections caused by C. albicans rare; mutations in four genes involved in the IL-17 immunity were shown to be responsible for CMCD so far. The genetic etiology remains unknown in about half the MSMD or CMCD patients and the cellular mechanisms critical to human anti-mycobacterial and/or anti-Candida immunity are poorly understood. In this collaborative research project we aim at identifying new genetic defects involved in IFN-γ or IL-17 immunity, and at characterizing their impact in the development and in the differentiation of T helper (Th) cell subsets, and in the function of naïve and memory $CD4^+$ T cells reactive against Mycobacteria or Candida. In particular, we are using high throughput cell-based screening assays to identify, quantify and functionally characterize memory $CD4^+$ T cells (Th1, Th2, Th1* and Th17) induced *in vivo* by Mycobacteria and Candida in patients with novel inborn errors of anti-mycobacterial and anti-fungal immunity. We are also studying the impact of the newly identified MSMD- and CMCD-causing genes in pathogen-induced human Th cell differentiation in vitro. The outcomes of this project should have considerable consequences in immunology, in terms of understanding anti-Mycobacteria and anti-Candida cellular immunity, as well as profound and broad medical implications, in terms of diagnosis and treatment of human mycobacterial and fungal diseases.

Collaborators: Jean-Laurent Casanova, Rockefeller University, New York (US), Anne Puel, Imagine Institute, Paris (FR), Alessandro Sette and Bjoern Peters, La Jolla Institute for Allergy and Immunology, La Jolla (US).

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Th2 cells limit the ability of Th17 cells to control C. albicans infection

Camilla Basso, Roberta Marzi, Luana Perlini and Federica Sallusto

Although normally a commensal, *Candida albicans* can become an opportunistic pathogen, causing insidious mucosal diseases as oropharyngeal and vulvovaginal candidiasis. While in healthy donors Th17 cells are the major player responding to *C. albicans* infection, in patients affected by chronic infection we observed an altered distribution of *C. albicans*-specific Th cell subsets, with a predominant Th1 and Th2 cell response. In order to study the adaptive immune response conferring long-term protection to *C. albicans* infection, we set up an in vivo model of vulvovaginal candidiasis (VVC) in Cd3e–/– immunodeficient mice. We demonstrated that only antigen-specific IL-17A+ Th17 cells were able to clear *C. albicans* infection. Interestingly, Th2 cells exert a wasting effect on reconstituted Cd3e–/– mice, affecting Th17 cell expansion and protection against *C. albicans* infection. Treatment with an antibody neutralizing the Th2-derived IL-4 rescues the long-term containment of *C. albicans* given by Th17 cells, suggesting a possible approach to cure recurrent and chronic candidiasis caused by imbalanced adaptive immunity.

Collaborators: Anne Puel, Imagine Institute, Paris (FR), Salomé LeibundGut and Burkhard Becher, University of Zurich (CH).

Sparber F. et al.
Cell Host Microbe. 2019, 25:389-403.

Type-17 immunity controls Malassezia-mediated skin infection
Corinne De Gregorio and Federica Sallusto
There is accumulating evidence that Malassezia spp involved in the development and/or exacerbation of various skin disorders including pityriasis versicolor, dandruff, seborrhoeic eczema and atopic dermatitis. The causal relationship between the fungus and these pathologies remains however unclear. To gain knowledge about the complex interplay between Malassezia and the skin immune system, our collaborator has established a novel experimental model of Malassezia skin infection in mice. This has allowed deciphering the cellular and molecular mechanisms that control the fungal growth on the skin. They found that Malassezia spp. selectively induce IL-17 and related cytokines. This response is key in preventing fungal overgrowth on the skin, as disruption of the IL-23-IL-17 axis compromises Malassezia-specific cutaneous immunity. Under conditions of impaired skin integrity, mimicking a hallmark of atopic dermatitis, the presence of Malassezia dramatically aggravates cutaneous inflammation, which again was IL-23 and IL-17 dependent. Consistent with these mouse data, we found a CCR6+ Th17 subset of memory T cells to be Malassezia specific in both healthy individuals and atopic dermatitis patients, whereby the latter showed enhanced frequency of these cells. Thus, the Malassezia-induced type 17 response is pivotal in orchestrating antifungal immunity and in actively promoting skin inflammation.

Collaborators: Salomé Leibundgut-Landmann, University of Zurich (CH), and Martin Glatz, University Hospital Zuric (CH).

Dominant and cryptic epitopes in Influenza HA identified by combination of T cell libraries and peptidomics
Antonino Cassotta, Roger Geiger, 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 to the development of immunological memory following vaccination. By combining antigenic stimulation of naïve and memory T cell libraries, T cell cloning, peptide mapping and TCR sequencing, we provide a comprehensive description of the clonal composition of human Th cell repertoire against HA. Furthermore, using mass spectrometry-based approach we define the MHC-II immunopeptidome of monocytes-derived dendritic cells and HA-specific B cells pulsed with recombinant HA. 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)

Specificity and cross-reactivity of T cells elicited by Zika virus infection
Antonino Cassotta and Federica Sallusto
Zika virus (ZIKV) and Dengue viruses (DENV) belong to the 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 of 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 infected by ZIKV develop antibodies and T cells that cross-react with DENV is unclear. In this study performed in the context of the EU financed project Zikalliance, we set out to dissect the level of cross-reactive immunity at the B- and T-cell level in response to ZIKV infection.

Collaborators: Lorenzo Zammarchi and Alessandro Bartoloni, Careggi Hospital, Firenze (IT) and Cristina Gagliardi and Daniela Peruzzu, Istituto Superiore di Sanità, Roma (IT).

Broadly reactive T cells against Enterobacteriaceae are present in the naive and enriched in the memory repertoire.

Antonino Cassotta, Daniela Latorre, Jérémie Goldstein, Greta Durini, David Jarrossay, Antonio Lanzavecchia and Federica Sallusto

Enterobacteriaceae are a large family of Gram-negative bacteria that includes both commensals and mutualistic or opportunistic pathogens. The latter can cause severe nosocomial infections, with sporadic outbreaks of multi-antibiotics resistant strains, thus being a major public health threat. Better understanding of immune defense mechanisms against Enterobacteriaceae and identification of relevant microbial antigens may yield new strategies for prevention and treatment of severe infections. In this study, we used sensitive experimental techniques to analyse Enterobacteriaceae-reactive CD4+ T cell repertoires in the blood of healthy donors and of patients with sepsis. This approach led to the isolation of Enterobacteriaceae-specific memory $CD4^+$ T cells, which were highly enriched in the $CCR6^+CXCR3^+CCR4^-$ T cell subset, expressed gut homing markers and produced IFN-γ, IL-17A and IL-22. This T cell population was absent in the blood of septic patients suffering from *K. pneumoniae* systemic infection. By combining *ex vivo* antigenic stimulation, single cell cloning and TCR Vβ sequencing, we found that a large fraction of memory T cell clones were broadly cross-reactive, responding to several Enterobacteriaceae species. Of note, up to half of the cross-reactive T cell clones reacted to the outer membrane protein A (OmpA) antigen. T cell clones cross-reactivity to Enterobacteriaceae species were also detected in the $CD4^+$ naïve compartment, although to a lesser extent than in the T cell memory compartment. These data point to the existence of epitope determinants conserved among different Enterobacteriaceae species and that are targeted by broadly cross-reactive T cells that are already present in the naïve pre-immune repertoire and are selected and become dominant in the memory repertoire.

Collaborators: Franca Baggi Menozzi, Istituto Cantonale di Microbiologia, Bellinzona (CH) and Cristina Gagliardi, Istituto Superiore di Sanità, Roma (IT).

Investigation of pathogen-cross-reactive TCRs at the clonal level

Antonino Cassotta, Jeremie Goldstein, Federico Mele, Antonio Lanzavecchia and Federica Sallusto

Nearly 20 years ago it was postulated that, given the huge amount of potential antigenic peptides that can be presented in the context of MHC molecules, T cells can provide an effective immune protection only if each T cell is capable of recognizing numerous peptide-MHC complex and is therefore cross-reactive. Heterologous immunity between related pathogens is common and historically has been exploited as a vaccination strategy, however little is known regarding the extent of cross-reactivity between phylogenetically distant pathogens. In this project, we are using a combined approach of in vitro T cell culture and TCR-seq to identify and isolate CD4+ T cells cross-reactive against human pathogens that have low genomic similarity, such as *Mycobacterium tuberculosis*, *Staphylococcus aureus* and Streptococcus pyogenes. With this approach, we will also be able to have information on the extent and quality of cross-reactivity and will attempt to identify the epitope targeted by the cross-reactive T cell clones. The identification of the antigenic determinants recognized by cross-reactive T cells may have important implications for the rational design of vaccines that could elicit cross-protection against a broad spectrum of human pathogens.

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 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. These T cells responded mostly to peptides from major allergens, had high functional avidity and correlated best with allergen specific IgE. Functional analysis revealed that CRTh2$^+$ T cells become IL-4, IL-5, IL9, and IL-13 quadruple producing cells in response to all allergens; while CCR10$^+$ cells produced primarily IL-22 in response to house dust mite. The unique cytokine profiles for each T cell subset are likely indicative of the complex etiology of the allergic response. Transcription factor analysis demonstrated that CRTh2$^+$ cells express GATA3 and not the Th9 transcription factor PU.1, confirming their Th2 phenotype. TCRβ repertoire analysis on allergic donors showed that HDM-reactive CRTh2 cells are dominated by few clonotypes highly expanded. This study identifies CRTh2$^+$ cells as the Th2 cell subset most involved in allergic responses and these cells have a previously undescribed, highly polyfunctional phenotype.

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, Sandra Jovic, David Jarrossay, Antonio Lanzavecchia and Federica Sallusto

Narcolepsy is a chronic sleep disorder caused by the loss of neurons that produce hypocretin. The close association with HLA-DQB1*06:02, evidence for immune dysregulation and increased incidence upon influenza vaccination together suggest that this disorder has an autoimmune origin. However, there is little evidence of autoreactive lymphocytes in patients with narcolepsy. We used sensitive cellular screens and detected hypocretin-specific CD4+ T cells in all 19 patients that we tested; T cells specific for tribbles homologue 2 (TRIB2) – another self-antigen of hypocretin neurons – were found in 8 out of 13 patients. Autoreactive CD4+ T cells were polyclonal, targeted multiple epitopes, were restricted primarily by HLA-DR and did not cross-react with influenza antigens. Hypocretin-specific CD8+ T cells were also detected in the blood and cerebrospinal fluid of several patients with narcolepsy. Autoreactive clonotypes were serially detected in the blood of the same, and even of different, patients, but not in healthy control individuals. These findings solidify the autoimmune aetiology of narcolepsy and provide a basis for rapid diagnosis and treatment of this disease.

Latorre D, Kallweit U, et al.
Nature. 2018, 562:63-68.

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Research Groups

Marcus Thelen
Signal Transduction

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

Cell migration and positioning is principal feature in the organization of multicellular organisms. During development of mammalian cells migrate along predefined gradients to find their destinations. 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 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. In addition, CXCR4 has unique signalling properties capable of promoting the sustained activation of intracellular signalling cascades, which is strictly dependent on the availability of extracellular CXCL12.
Locally produced chemokines are usually presented on the surface of neighbouring 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. An important aspect for the maintenance and local confinement of gradients is the requirement of sinks in apposition to the source of attractant. Atypical chemokine receptors (ACKRs) were recently defined as a separate group of receptors structurally related to typical chemokine 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 (formerly CXCR7 or RDC1) 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 its cognate receptor 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 signalling 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 signalling through G-proteins, ACKR3 may use biased signalling 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.

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Signalling of CXCR4 and ACKR3

Sabrina Casella, Sylvia Thelen and Marcus Thelen

G-protein coupled receptors (GPCRs) can display a signalling bias depending on the ligand and the local environment. Such signalling 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.

We use yellow fluorescent protein split variants (denotes Y1 and Y2) in bimolecular fluorescence complementation to reveal functional ACKR3 dimers in transfected cells. Figure 1 shows that ACKR3-Y1 and ACKR3-Y2 dimerize and actively scavenge CXCL12. Furthermore, using the APEX2-labeling procedure we found limited interactions of ACKR3 with arrestins. The data are in agreement with rapid receptor internalization in cells devoid of arrestins. The APEX2 protocol was developed in collaboration with the ETH Zurich (Wollscheid/Milani).

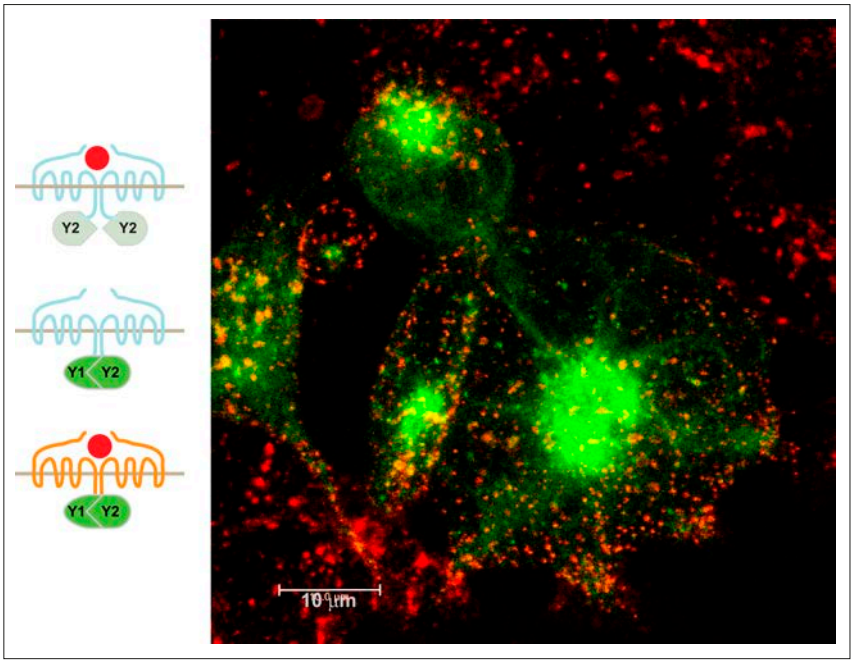

Figure 1.
Bimolecular fluorescence complementation (BiFC) mediated by ACKR3. Cells stably expressing ACKR3-Y2 were transiently transfected with ACKR3-Y1 and exposed to the ACKR3-specific chimeric chemokine CXCL11_12. ACKR-Y2/ ACKR3-Y1 appear in green and when bound to the chimeric chemokine (red) light up in orange.

ACKR3 expression in B cells

Egle Radice, Rafet Ameti and Marcus Thelen

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 cells 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. Inhibition of ACKR3 was shown to cause the distortion of the microarchitecture. Recent investigations from the laboratory demonstrate that expression of ACKR3 on B cells is required to establish the important physiological niche of the marginal zone. Moreover, expression of ACKR3 defines two phenotypically, transcriptionally and functionally distinct, equal-sized populations of mouse MZ B cells. Current investigations aim in understanding the role of ACKR3 for the marginal zone B cell-mediated immune responses.

Functional role of ACKR3 in B cells.

Expression of ACKR3 on primary human and mouse B cells is now established. We previously showed a functional expression 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. 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 in different vaccination models in wild type mice and animals with conditional deletion in B cell compartments.

In vivo studies of ACKR3 function

Rafet Ameti, Serena Melgrati, Sylvia Thelen and Marcus Thelen

In general, G-protein coupled receptor (GPCR) detection on live cells can be difficult. In mice, the lack of suitable antibodies often impedes monitoring of the expression of chemokine receptors on leukocytes. We generate fluorescent-labelled chemokines to measure receptor expression and activity. Recombinant chemokines are expressed 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 labelling with fluorescent-labelled Coenzyme A. After proteolytic removal of the N-terminus, chemokines undergo a final purification step by reversed-phase HPLC. Chemokine preparations can essentially be labelled with any fluorescent dye conjugated to Coenzyme A. To reveal in vivo scavenging activity of ACKR3 we constructed a fluorescent labelled chimeric chemokine CXCL11_12 that selectively binds to human and mouse ACKR3, but not to the typical receptors CXCR3 and CXCR4 (Figure 2).

Expression of ACKR3 in the spleen sinusoids.

Using the ACKR3$^{+/GFP}$ reporter mouse we observed a marked expression of ACKR3 on spleen sinusoids. Injection of the CXCL11_12 chimera in vivo demonstrates marked scavenging activity by the sinusoids, indication that these structures markedly contribute to the elimination of CXCL12 from the circulation (Figure 3).

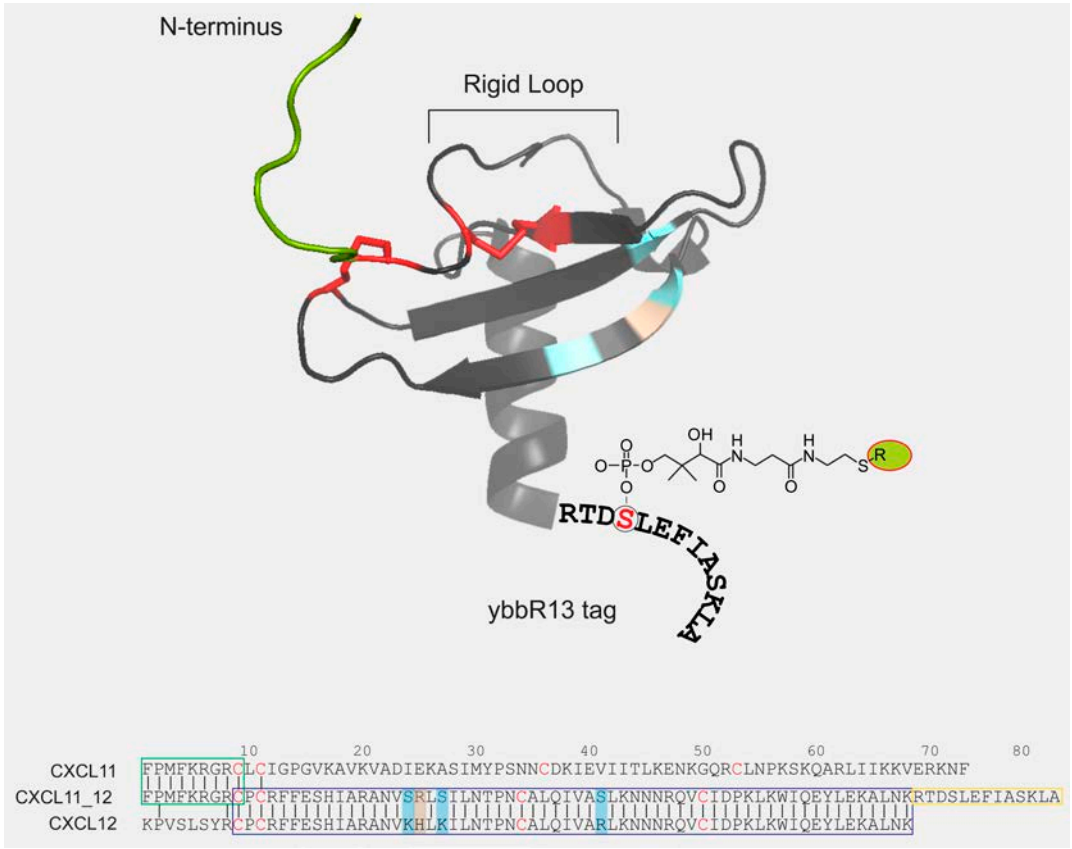

Figure 2.
Schematic structure of the chimeric chemokine CXCL11_CXCL12_Gag-_ybbR13_labeled with fluorescent dyes.

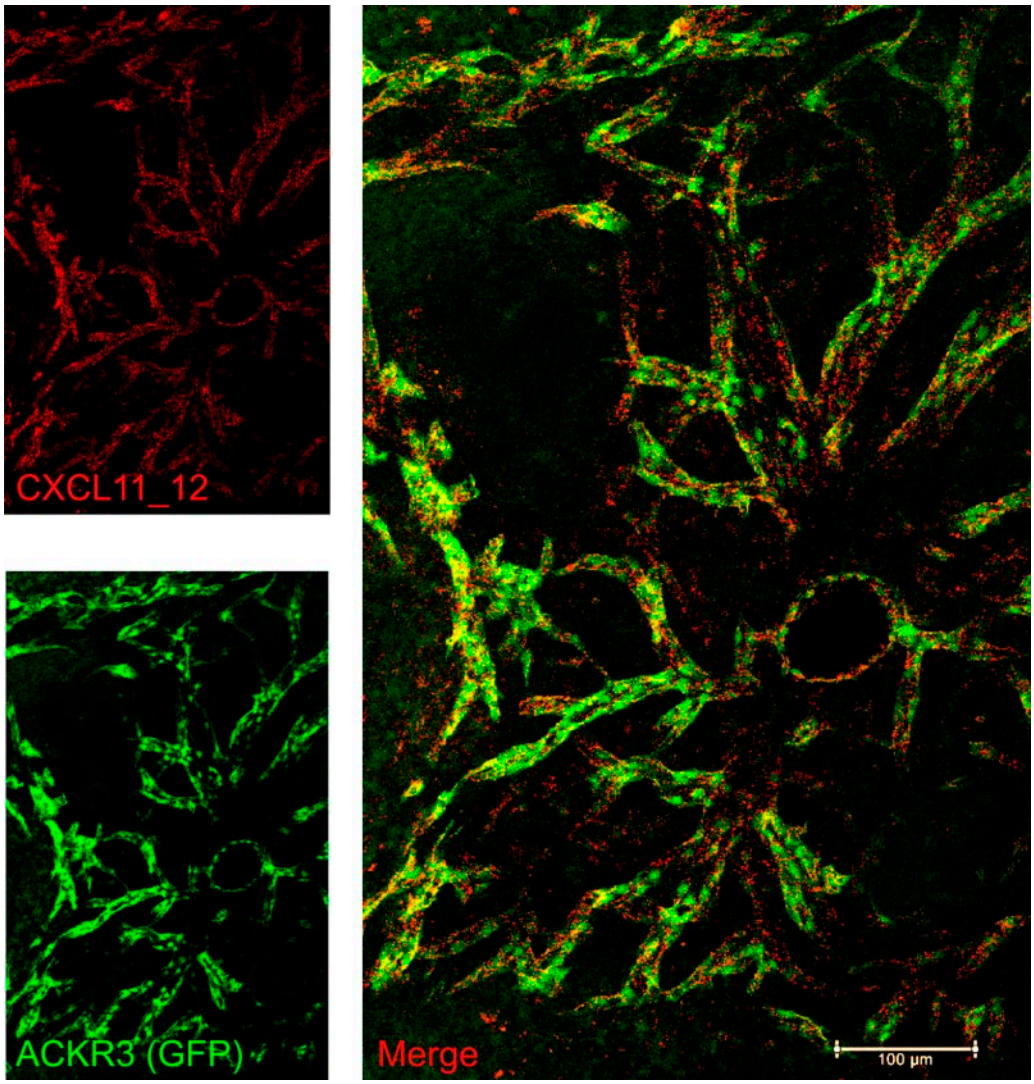

Figure 2.
CXCL11_12 marks ACKR3 scavenging. Detail of a spleen section from a ACKR3$^{+/GFP}$ (green) reporter mouse injected with the chimeric chemokine CXCL11_CXCL12_Gag-_ybbR13_labeled with Alexa647 (red).

Role of ACKR3 in cancer

Paola Antonello, Sabrina Casella, Egle Radice, 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 tumours is often associated with a good prognosis. By contrary dissemination of the primary tumour leads to a poor prognosis. Dissemination and organ infiltration of lymphomas requires migration of tumour 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 tumours of mesenchymal cell origin. We have shown the expression of ACKR3 on B cells, in particular on plasmablasts of GCs. 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, such as the brain (Figure 4). We are currently investigating the mechanism by which ACKR3 enhances tissue infiltration.

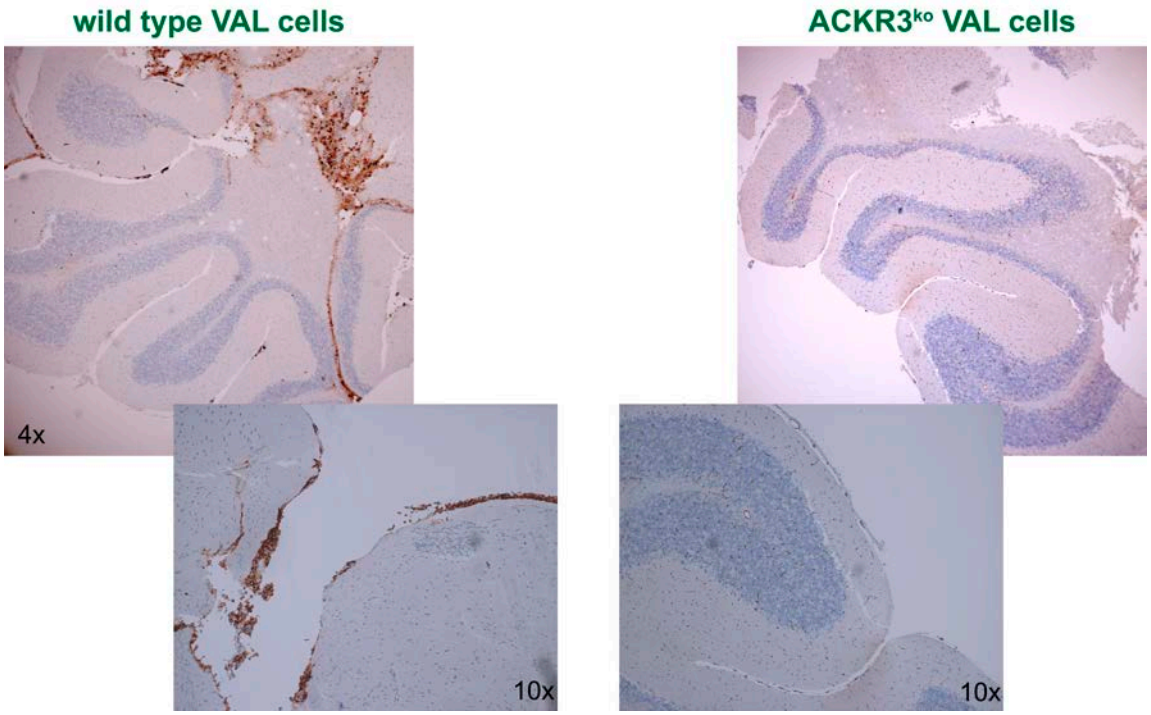

Figure 4.
Brain infiltration of DLBCL Val cells in a diffused xenograft model. VAL DLBCL expressing ACKR3 (wild type) and a counterpart with CRISP/Cas9-mediated elimination of ACKR3 (ACKR3^{ko}) were injected i.v. Tissue infiltration was determined after 3-4 weeks.

Funding

Swiss National Science Foundation
The Atypical Chemokine Receptor ACKR3: Function in vivo in Immune Responses and Coupling to Signal Transduction Pathways
310030_163336 / 2015 – 2018

Swiss National Science Foundation
Function of ACKR3 in immune cell trafficking in lymphoid organs
310030 182727 / 2018 – 2022

Swiss National Science Foundation
Interplay of classical and atypical chemokine receptors in immune cell trafficking and dynamic microarchitecture of the secondary lymphoid organs
Sinergia CRSII3_160719 responsible applicant, together with C. Halin, D. Legler and A. Rot
2015-2019

Novartis Foundation
Role of ACKR3 expression on follicular dendritic cells
2017-2018

Swiss Cancer League
The function of ACKR3 in B-cell lymphoma tissue invasion
2018-2021

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Research Groups

Mariagrazia Uguccioni
Chemokines in immunity

Mariagrazia Uguccioni received a degree in Medicine from the University of Bologna (Italy) where she specialized in Haematology in 1994. From 1993 to 2000 she was a member of the Theodor Kocher Institute, University of Bern (Switzerland), since 2000 she is group leader at the IRB, and deputy director since 2010. In 2016, she has been nominated extraordinary Professor at the Medical faculty of Humanitas University (Italy). For her studies on the relevance of chemokines in human pathology, she was elected Member of the Bologna Academy of Science in 2009. 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 clarifying the mechanisms leading to chemokine synergism in leukocytes, and the modifications occurring in leukocytes from patients with chronic inflammatory conditions, which result in dysfunction of chemokine receptors, and maintenance of a proinflammatory microenvironment.

Research Focus

The research interest of the laboratory 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.

Cell trafficking to tissues, in homeostasis and in pathology is guided by chemokines and activation of chemokine receptors. The binding of most chemokines to their cognate seven-transmembrane-domain, G protein-coupled receptors, results in intracellular cell signalling transduction associated with the activation of several pathways. Nonetheless, the activity of chemokines is not only determined by the binding to a chemokine receptor, but is regulated by several mechanisms that control the final cellular response. In recent years, we have dissected the complexity of the regulation of the chemokine system at the level of the agonists and of the receptors, and shown how the dysfunction of chemokine receptors occurring in the chronic activation of the immune system can be reverted to restore function and homing potential of leukocytes. The group is now studying the activity of T cells in individuals and patients characterized by a sustained activation of the immune system, in order to identify those populations that present a defect in chemokine receptor activation.

During the inflammatory response, from the onset to the chronic phase, the release of exogenous agents (e.g.: bacterial and viral products) and the induction of endogenous mediators (e.g.: cytokines, chemokines and DAMPS) contribute to the recruitment of circulating leukocytes to the inflamed site. We have described a 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, we still know very little about the capacity of non-ligand molecules, other than chemokines, to synergize with chemokine agonists.

The study of chemokine activity modulation and of chemokine receptor dysfunctions in disease might shed new light on novel pharmacological interventions aimed at favouring resolution of inflammation, or restoring chemokine receptor activities in persistent inflammation and infections.

Team

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Chemokines: Structure/Function Studies

Gianluca D'Agostino, Valentina Cecchinato, Gabriela Danelon, Ajay Panakal, Edisa Pirani 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. Moreover, we have described chemokines that can act in synergism with chemokine receptor agonists, forming heterocomplexes able to induce functional responses at lower agonist concentration.

A thorough study mapping the chemokine heterocomplexes opens the debate on the relevance of the multitude of heterocomplexes found *in vitro*. Nonetheless, our group and others have shown that some of the identified complexes could strongly change the activity of chemokine receptors *in vivo*.

Based on the expression in pathological tissue samples, we have identified novel pairs of chemokines and molecules to be test for a possible activity in tuning chemokine functions.

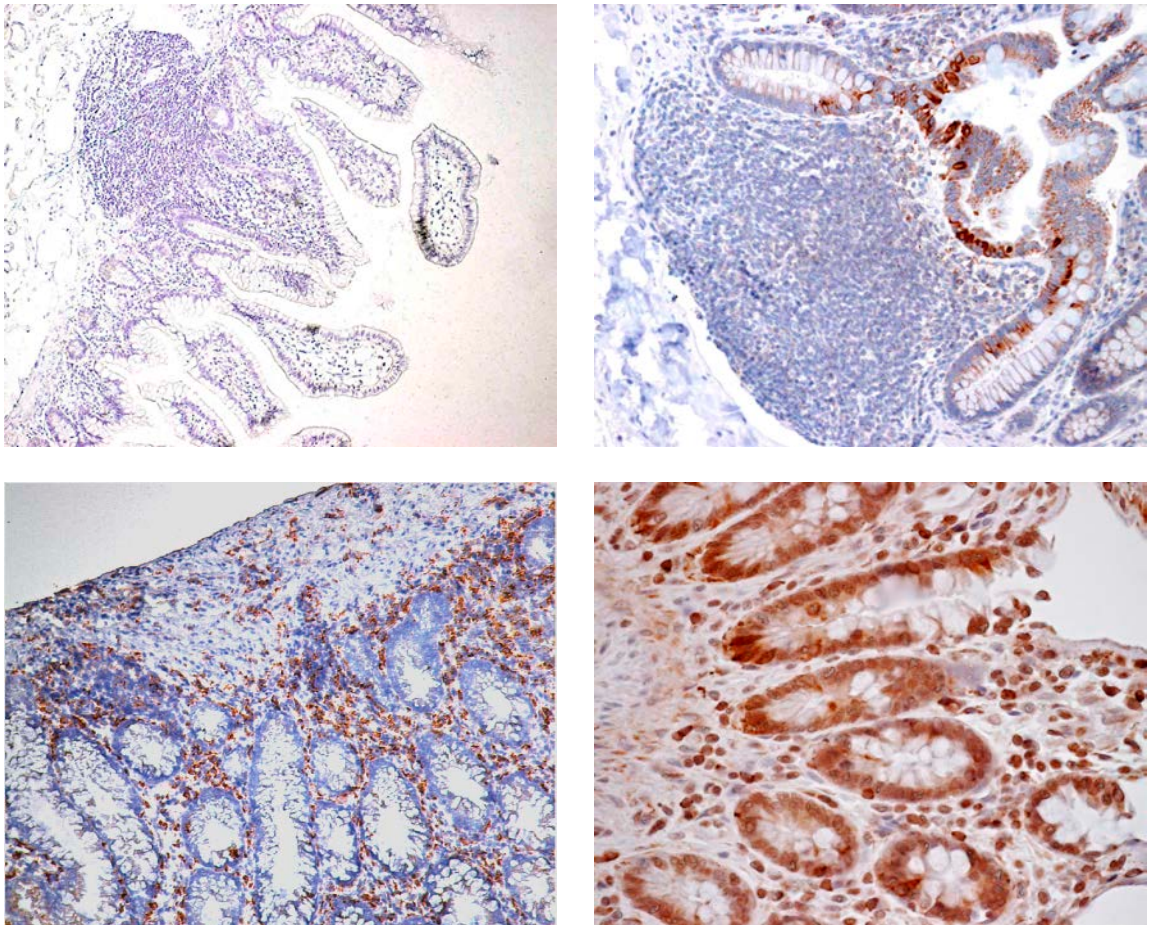
The identified chemokines and molecules are tested on selective chemokine receptors with a specific function on human primary leukocytes. This allows the identification of heterocomplexes able to modulate traffic in physiology or in pathological conditions in which cell migration, in the affected organ, sustains the disease.

Together with the IRB group of Andrea Cavalli, we have continued the analysis of the CXCL12/HMGB1 heterocomplex by molecular dynamic (MD) simulations to elucidate the effect of the disulfide bond on the structure and dynamics of HMGB1. The results of the MD simulations corroborate the data obtained in vitro, and show that the presence or lack of the disulfide bond between Cys23 and Cys45 modulate the conformational space explored by HMGB1, making the reduced protein more suitable to form a complex with CXCL12.

G. D'Agostino et al.
Frontiers in Immunology 2018, 9: article 2185

P.J. Collins et al.
FASEB J. 2017, 31:3084-3097

Figure 1.
Chemokine and HMGB1 expression in human intestine. A: chemokine mRNA detected by in situ hybridization. Positive cells are shown in black. B-D: expression of different chemokines (B and C), or inflammatory molecules (D) detected by immunohisto-chemistry. Positive cells are shown in brown. Unpublished data.



Responses to chemokines in chronic immune activation

Valentina Cecchinato, Gianluca D'Agostino, Gabriela Danelon, Ylenia Silvestri and Mariagrazia Uguccioni

Chemokine synergy-inducing molecules are emerging as regulating factors in cell migration. The alarmin HMGB1, in its reduced form, can complex with CXCL12 enhancing its activity on monocytes via the chemokine receptor CXCR4, while the form containing a disulfide bond, by binding to TLR4, initiates a cascade of events leading to production of cytokines and chemokines. So far, the possibility that the CXCL12/HMGB1 heterocomplex could be maintained in a chronic inflammatory microenvironment was debated, due to the release of reactive oxygen species. We have demonstrated that in patients with active Rheumatoid Arthritis, the activation of mechanisms counteracting the oxidative stress in the extracellular compartment preserves HMGB1 in its reduced form, and contributes to fuel the influx of inflammatory cells.

Not only inflammatory molecules produced in pathological conditions can modulate chemokine activities, but also changes in the ability of chemokine receptors to modulate cellular responses can lead to leukocyte dysfunctions.

In HIV 1-infected individuals, $CD4^+$ T-cell repopulation of the gut is rarely achieved even in patients receiving clinically effective anti-retroviral therapy (ART). Alterations in the integrity of the mucosal barrier have been indicated as cause for chronic immune activation and disease progression. We have shown that persistent immune activation causes impairment of lymphocytes to respond to chemotactic stimuli, thus preventing their trafficking from the blood stream to peripheral organs. We have demonstrated that this defect is due to hyper-activation of cofilin and inefficient actin polymerization, and that cytoskeleton remodeling with okadaic acid restores lymphocyte migration in response to chemokines, both *in vitro* and *in vivo*. This study calls for novel pharmacological approaches in those pathological conditions characterized by persistent immune activation and loss of trafficking of T-cell subsets to niches that sustain their maturation and activities. In addition, it encourages us to extend our studies to cohorts of patients with different type of chronic inflammatory conditions, to identify those populations that would benefit of novel pharmacological approaches for restoring T cell responses to chemotactic cues.

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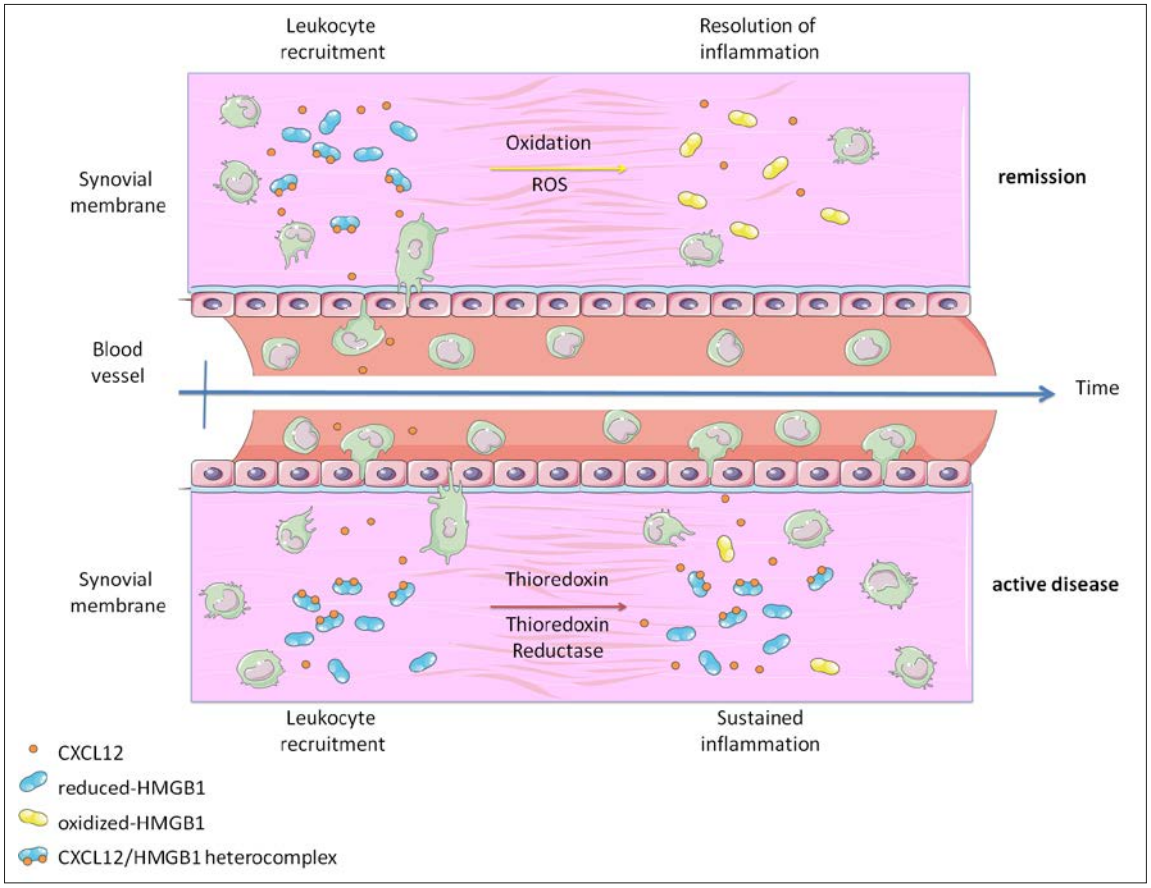

Figure 2.
Graphical representation on how the CXCL12/HMGB1 heterocomplex is maintained in active Rheumatoid Arthritis thanks to the activity of the Thioredoxin system.
From: Cecchinato et al., Frontiers in Immunology, 2018.

Responses to chemokines in cancer cells and effects of therapy

Valentina Cecchinato, Gianluca D'Agostino, Gabriela Danelon, Edisa Piarani, and Mariagrazia Uguccioni

Breast cancer is the most common diagnosed tumour and represents the second cause of death in women. The metastatic potential of breast cancer cells has been strongly associated to the overexpression of the chemokine CXCL12, and the activity of its selective receptor CXCR4.

Retrospective and clinical studies on patients' cohorts undergoing cancer surgery suggested that preoperative use of regional anaesthesia and the use of local anaesthetic agents might improve the outcome. As local anaesthetics are known to modulate the activity of different G proteins, we investigated whether lidocaine at clinical concentration can modulate the CXCR4 responses induced by CXCL12. We have shown that lidocaine significantly inhibits CXCR4 signalling, impairing the essential cascade of cytoskeleton remodelling, leading to a reduced motility of cancer cells, which is not associated to modulation of adhesion molecules.

Lidocaine can thus block a potent pathway involved in cancer progression and metastasis, and this study opens the way for further investigations in the field of anaesthesiology that would also take into account the activity of lidocaine on the cells of the immune system, which can further contribute to improve cancer outcome. Studies performed *ex vivo* on tumour and infiltrating cells isolated after surgical removal of the tumour from patients treated with lidocaine are in progress to confirm the data obtained so far, and to clarify a possible novel role for local anaesthetics in cancer therapy.

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Funding

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

San Salvatore Foundation
The role of chemokine synergy-inducing molecules in controlling the tumour microenvironment, cell migration and metastasis
2013-2019

Ceschina Foundation
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2016-2021

Fondazione per la Ricerca sulle Malattie Virali
The role of chemokines in tuning the inflammatory responses during hepatitis c virus infection
2016-2017

Fondazione Rocca
The role of a novel chemoattractant produced by macropahges
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Publications

Redox-Mediated Mechanisms Fuel Monocyte Responses to CXCL12/HMGB1 in Active Rheumatoid Arthritis.
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J Immunol. 2017; 198:184-195.

Research Groups

Luca Varani
Structural Biology

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 IRB (Bellinzona, CH). The main research activity involves the characterization of interactions between pathogens and antibodies, molecules of the immune system capable of curing and protecting from illness. His group strives 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 group managed to rationally modify an existing antibody utilizing, for the first time, only computational tools, thus increasing its ability to neutralize Dengue virus by 50 fold. It also completed one of the rare studies on antibody flexibility, showing how the antibody interferes with the pathogen altering its flexibility. More recently, the same approach led to the design of a bispecific antibody capable of preventing Zika virus escape mechanisms and another bispecific that can cure prion disease even when signs of neurodegeneration are already evident in cellular assays. The group has had recent high impact publications in journals such as *Cell*, *Science*, *Nat. Mol. Biol.* and *PNAS*. 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 understand how antibodies, molecules of the immune system, can recognize and defeat pathogens. This information allows us to optimize existing antibodies or design new ones. Understanding how antibodies function also has implications for vaccine design, since vaccines aim to generate a protective antibody response in the individual. Discovering which part of a pathogen is recognized by the most effective antibodies allows identifying and blocking the key parts of the pathogen itself. We work on rare diseases that do not attract investment from pharmaceutical companies such as Dengue and Zika, Prion diseases or rare forms 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 fatal neurodegenerative disease with no cure and still poorly understood. Our group studies the function and three-dimensional atomic structure of antibodies with 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 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 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 in a given pathogen, 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 immunotherapy strategies. Comparing the binding of several different antibodies to related Ags should also further our understanding of general principles of recognition.

We employ a highly multidisciplinary approach to discover i) the atomic structure of an antibody bound to its antigen; ii) the biophysical properties of the interaction and iii) the cellular and biological function of the antibody (e.g. inhibition of cellular receptor binding by the pathogen, membrane fusion etc.). We are one of the very few research groups in the world, and only one in Switzerland, that approach antibodies with solution Nuclear Magnetic Resonance, whose main advantage is the possibility to characterize not only the structure but also the residue-level dynamic movement of molecules. We exploit the benefit of experimentally validated computational simulations to answer questions not easily approached exclusively by experimental methods.

We then use all the above information to rationally modify existing antibodies or design new artificial molecules such as single chain or bispecific antibodies with the aim to either improve or achieve new function.

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 rivalling that of malaria. ZIKV has recently seen epidemic outburst and is 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 favourable 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. Fig 1 illustrates the advantage of NMR validated computational simulations that significantly improve the accuracy achieved by cryo-EM alone.

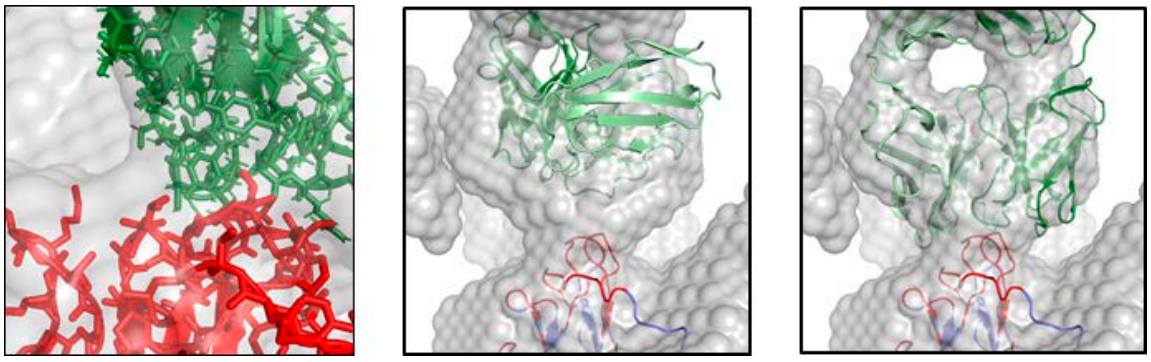

Figure 1.
NMR guided computational simulations provide a three-dimensional atomic structure of antibody-antigen complexes increasing the accuracy of cryo-EM structures. Left: cryo-EM structure of ZKA190 antibody in complex with Zika Virus; unrealistic steric clashes between Ab (green) and virus (red) are evident. Middle: refinement of the cryo-EM structure; the large gap between Ab and Ag is unusual and does not fit the electron density (grey) well; Right: NMR guided computational structure (green and red cartoon) superimposed on the cryo-EM electron density (grey).

In a work published on *Cell* we characterized the structure and mechanism of action of ZKA190, an extremely potent antibody capable of protecting from Zika Virus infection. We showed, however, that the virus can readily escape the antibody response via frequent and easily achievable mutations. In order to avoid the problem we produced a bispecific antibody, combination of ZKA190 with a second molecule having a different epitope, and showed that it can i) neutralize the viral escape mutants generated in response of single antibody treatment and ii) prevent the formation of further escape mutants.

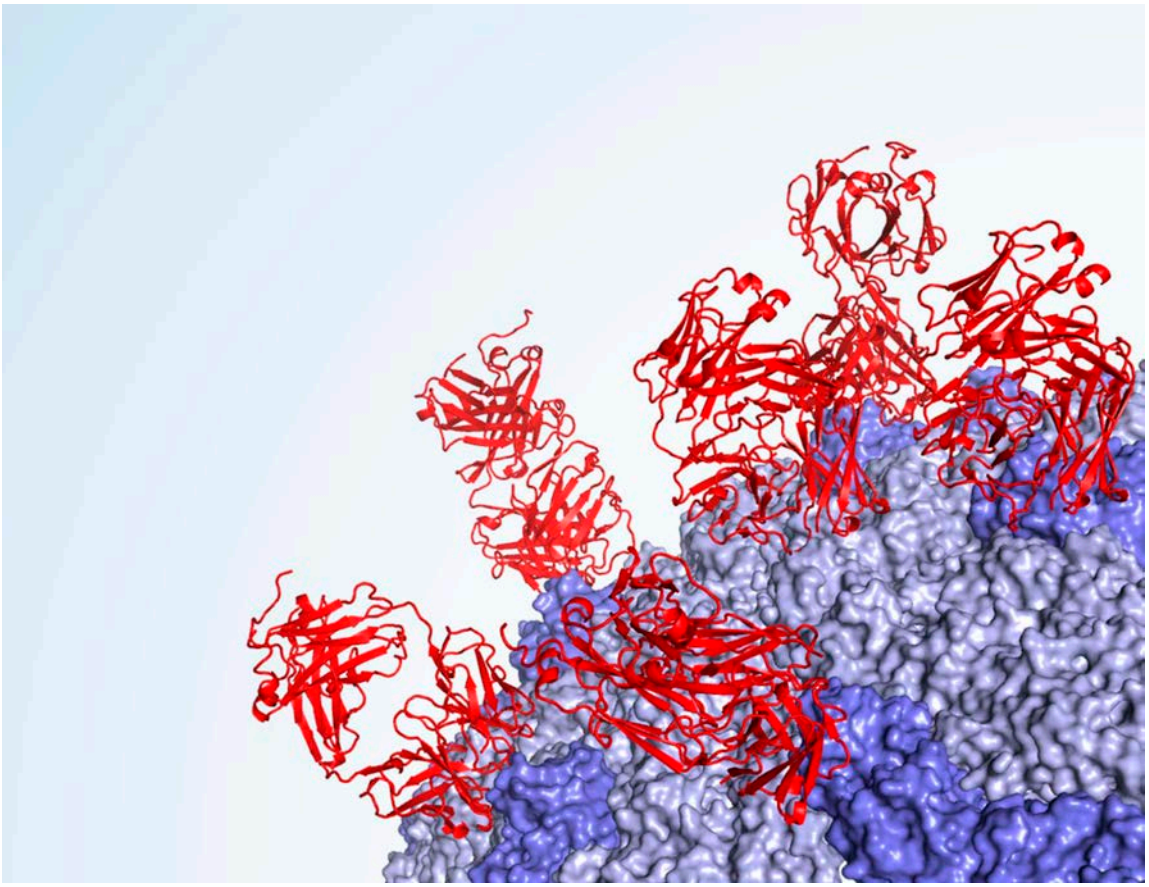

Figure 2.
Cartoon representation of antibody ZKA190 (red) on the cryo-EM structure of Zika virus (blue). The antibody potently neutralizes all circulating strains of ZIKV preventing the conformational rearrangement of the surface proteins required for viral fusion and infection.

Antibodies to investigate and cure prion diseases

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 (PrP), 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.

Antibodies are considered a valid strategy to defeat prion disease, especially its hereditary forms, in a similar way to the anti-Alzheimer antibodies currently in clinical trial. One major hurdle, however, is that once the illness becomes apparent it is often too late to provide effective treatment. Recent years saw a surprising and worrying discovery: antibodies against the so-called Globular Domain (GD) of PrP, such as the one hinted for clinical trial, can actually cause PrP-dependent neurotoxicity in the absence of prion infection, suggesting that utmost care should be taken before going into clinical trial. No toxic antibody against the Flexible Tail (FT) was found, so it was suggested that these should be used for therapy.

We hypothesized the presence of "toxicity triggering sites" (TTS) in the GD of PrP. In our model, binding to such a site by prion, toxic antibodies or other factors would "push" PrP, and the FT in particular, into a toxic conformer leading to cellular toxicity. According to this model, blocking the TTS would hinder prion infection by preventing prion molecules from interacting with it. To this avail, we designed a bispecific antibody formed by the neurotoxic POM1 antibody (that binds to the TTS) and POM2, an antibody that binds the FT and prevents it from achieving a toxic conformation. The resulting bispecific is not toxic and, most importantly, potently protects from prion infection in cellular models even when signs of neurodegeneration are already evident. Notably, a cocktail of the two single antibodies (POM1 and POM2) does not protect from prion whereas the bispecific does. Simultaneous targeting of GD and FT, we conclude, is a better strategy for immunotherapy for prion (and other neurodegenerative diseases) than what is currently being pursued by other research groups.

We were also able to show that the bispecific prevents the formation of relatively small, toxic soluble oligomers. These, rather than larger insoluble fibrils commonly associated with prion, cause toxicity in cellular models. The idea that toxicity arises from soluble oligomers capable of travelling through cells, rather than larger fibrils, is accepted in neurodegenerative diseases like Alzheimer or Tauopathies but had never been shown in prion protein.

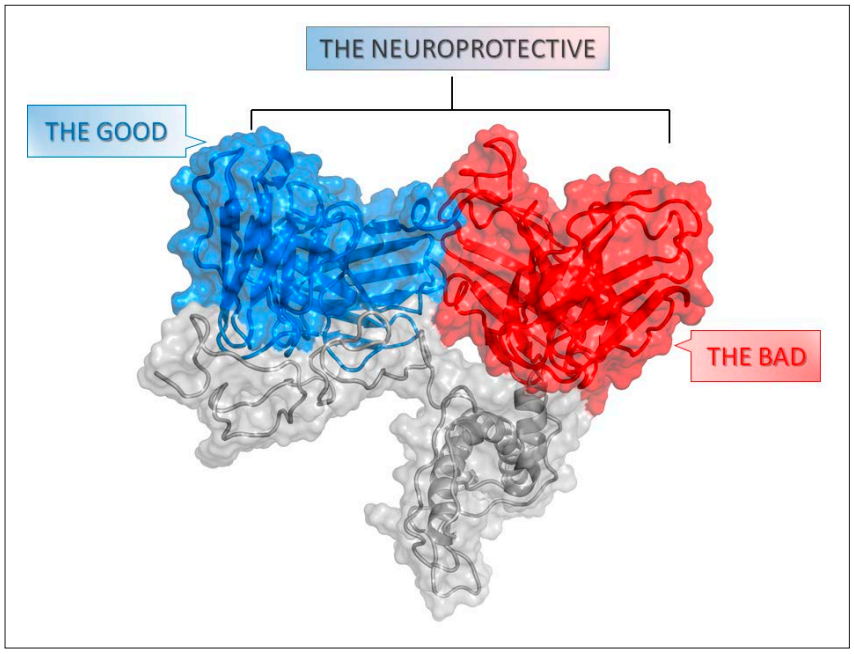

Figure 3.
we engineered a bispecific antibody capable of curing prion diseases in cellular models even if administered when signs of neurodegeneration are already evident. The bispecific is formed by i) POM1, a toxic antibody (red) that binds to a Toxicity Triggering Site in the Globular Domain of prion protein (grey) thus preventing prion from interacting with it; ii) a second antibody (blue) that prevents POM1 from triggering toxicity.

Targeting Acute Myeloid Leukemia with antibodies

Marco Bardelli, Luca Simonelli, Mattia Pedotti and Luca Varani

The immune system can readily distinguish pathogens (non-self) from our own molecules (self), leading to neutralization of the former. This is not generally true for cancer, whose cells are "self" but responsible for disease. One way to bypass the problem is to generate artificial antibodies capable of recognizing protein markers on the surface of cancer cells. Such "targeting" antibodies can then be exploited for the delivery of drugs such as chemotherapy agents (so called Antibody Drug Conjugates). In a relatively recent development, antibodies are also fused to engineered cells of the immune system, conferring them the ability to recognize and destroy cancer cells. These are generally referred to as CAR, Chimeric Antigen Receptor.

Unfortunately, the above-mentioned protein markers are not exclusively expressed on cancer cells but are also present on healthy cells, albeit at lower expression level. This leads to the "on target off tumour" problem, leading to the undesired destruction of healthy cells.

We investigated how tuning the antibody recognition of its target can help discriminating between cancer and healthy cells in Acute Myeloid Leukemia (AML), a disease with poor prognosis (26% survival rate at 5 years) and high recurrence of relapses due to the presence of Leukemic Stem Cells that are not affected by normal chemotherapy drugs. Rational engineering of antibody mutants with different binding affinity allowed 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; an approach that had never been tried before in the literature.

We showed that lowering the antibody affinity results in better discrimination between leukemic and healthy cells (increased therapeutic safety) without adversely affecting the killing of target cells from human donors (therapeutic efficacy). Efficacy was affected, instead, by the number of antibody molecules present on each CAR T-cell. Overall, the conclusion is that when designing CAR T-cells (against AML) resources are better devoted to the optimization of antibody expression levels on CAR cells rather than on the search for very low affinity antibodies which, instead, are usually sought after.

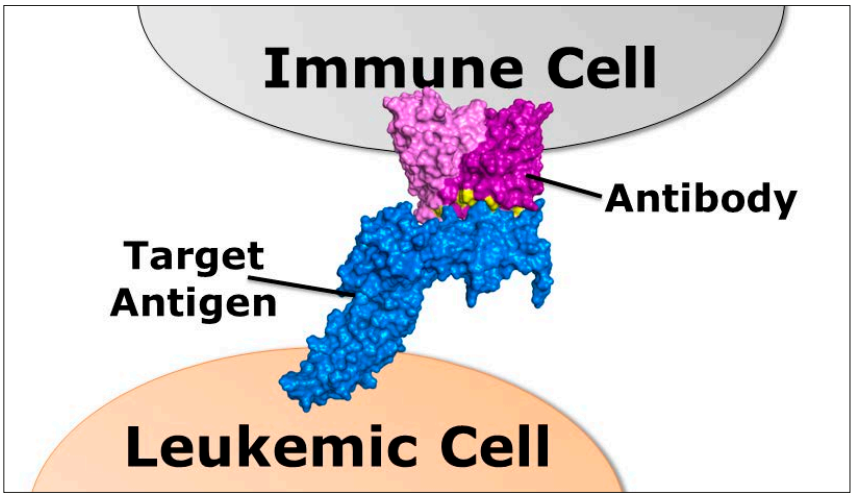

Figure 4.
Lowering the affinity for the target antigen (blue) of an antibody (purple) fused to a CAR T-cell allows discrimination between leukemic and healthy cells, resulting in increased safety without decreasing efficacy.

Funding

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)

Novartis Foundation
Structural and dynamic investigation of Prion protein antibody-mediated toxicity and protection
2017-2018

Interreg
Leuciti: Collaborazione tra enti di ricerca, piccola media impresa e centri ospedalieri per lo sviluppo di nuove terapie anti leucemia basate su anticorpi e nanoparticelle
2018-2020

Collaborations

CSCS, Swiss Supercomputer Center
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Humabs (CH)

Cerbios SA (CH)

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Università del Piemonte Orientale (IT)

Lok Shee Mei
Duke-NUS Medical School (SG)

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Core facilities


eppendorf
INTUITION

Gene Expression and Protein Purification Platform

Focus

The aim of the IRB Gene Expression and Protein Purification Platform (GEPP) is to provide training to IRB researchers seeking advice in protein expression, purification and characterization. The group possesses specific expertise in host-pathogen interactions and vaccine design. Isothermal Titration Calorimetry (ITC) and Circular Dichroism (CD) spectroscopy instruments are freely accessible after training. The team is also interested in collaborative efforts on infectious diseases with unmet medical need and collaborate with different IRB groups.

In addition to its training purpose, the team is actively pursuing research activities by obtaining competitive grants. The team specialized in vaccine-design and protein interaction uses biocomputing (Foglierini et al., 2019), biophysics, biochemistry (Tan et al., 2018) and cell-based approaches. Recent achievements of the team include the dissection of the molecular mechanisms of the human cytomegalovirus (HCMV) entry, characterization of its structural aspects (Kabanova et al., 2016; Martinez-Martin et al., 2018) and generation of an RSV nanoparticle-based vaccine (Marcandalli et al., 2019). The team aim is to investigate host-pathogen interactions at the molecular level and use nanoparticle-based vaccine design, structural biology and protein engineering to fight infectious agents.

Research projects

Neuropilin-2 is a Central Viral Receptor for Human Cytomegalovirus.

Jessica Marcandalli, Michela Perotti, Mathilde Foglierini, Antonio Lanzavecchia and Laurent Perez

Characterizing cell surface receptors mediating viral infection is critical for understanding viral tropism and developing antiviral therapies. Nevertheless, due to challenges associated with detecting protein interactions on the cell surface, the host receptors of many human pathogens remain unknown. We built a library consisting of most single transmembrane human receptors and implemented a workflow for unbiased and high-sensitivity detection of receptor-ligand interactions. We applied this technology to elucidate the long-sought receptor of human cytomegalovirus (HCMV), the leading viral cause of congenital birth defects. We identified neuropilin-2 (Nrp2) as the receptor for HCMV-pentamer infection in epithelial/endothelial cells and uncovered additional HCMV interactors. Using a combination of biochemistry, cell-based assays and electron microscopy, we characterized the pentamer-Nrp2 interaction and determined the architecture of the pentamer-Nrp2 complex.

Collaborators:
N Martinez-Martin, CS Huang, CP Arthur, H Ho, AM Dosey, S Shriver, J Payandeh, A Leitner, C Ciferri.

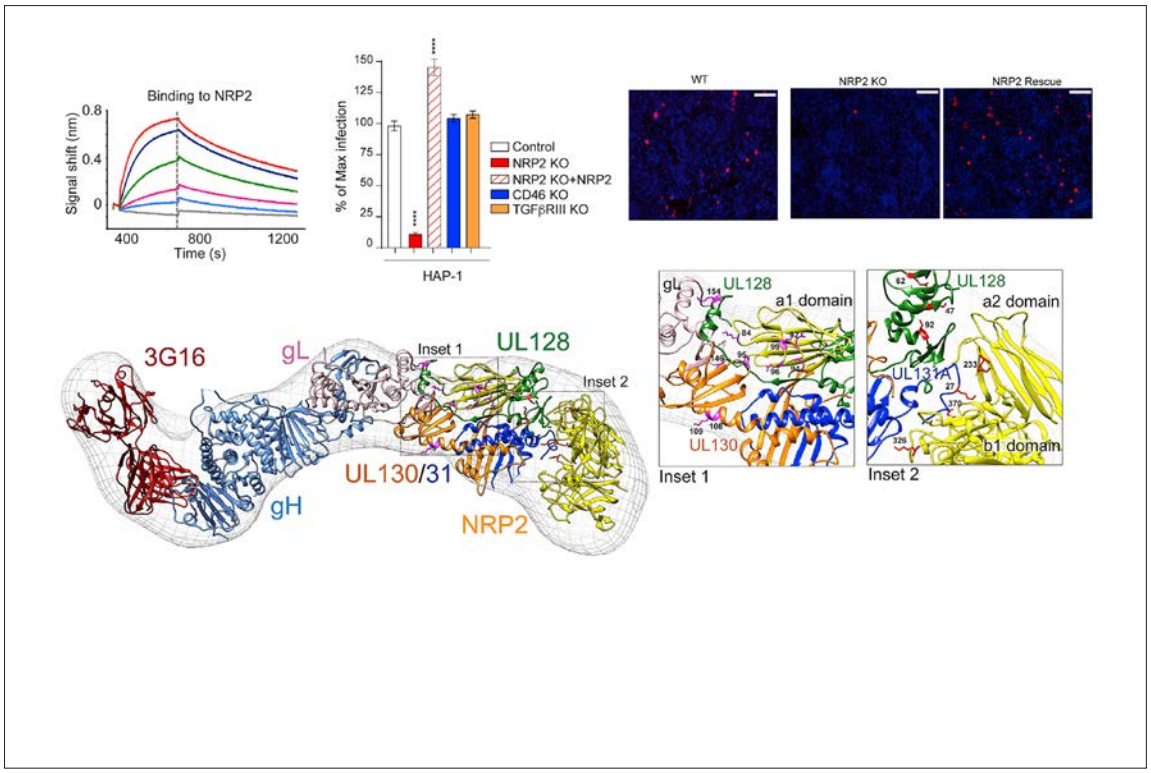

Figure 1.
Neuropilin-2 is a central viral receptor for Human Cytomegalovirus.
Receptor validation by bio-layer interferometry, cell-based assay using CRISPR/Cas9 methodology and electron microscopy combined with cross-linking mass spectrometry.

Vaccine for Respiratory Syncytial Virus using Designed Protein Nanoparticle.
Jessica Marcandalli, Michela Perotti, Antonio Lanzavecchia, Federica Sallusto and Laurent Perez
Respiratory syncytial virus (RSV) is a worldwide public health concern for which no vaccine is available. Elucidation of the prefusion structure of the RSV F glycoprotein and its identification as the main target of neutralizing antibodies, have provided new opportunities for development of an effective vaccine. We described the structure-based design of a self-assembling protein nanoparticle presenting a prefusion-stabilized variant of the F glycoprotein trimer in a repetitive array on the nanoparticle exterior. The two-component nature of the nanoparticle scaffold enabled the production of highly ordered, monodisperse immunogens that display DS-Cav1 at controllable density. In mice and nonhuman primates, the nanoparticle immunogen induced neutralizing antibody responses up to 10-fold higher than trimeric DS-Cav1.

Collaborators:
B Fiala, S Ols, W Schueren, J Snijder, E Hodge, M Benhaim, R Ravichandran, L Carter, W Sheffler, L Brunner, M Lawrenz, P Dubois, KK Lee, D Veesler, CE Correnti, LJ Stewart, D Baker, K Loré, NP King.

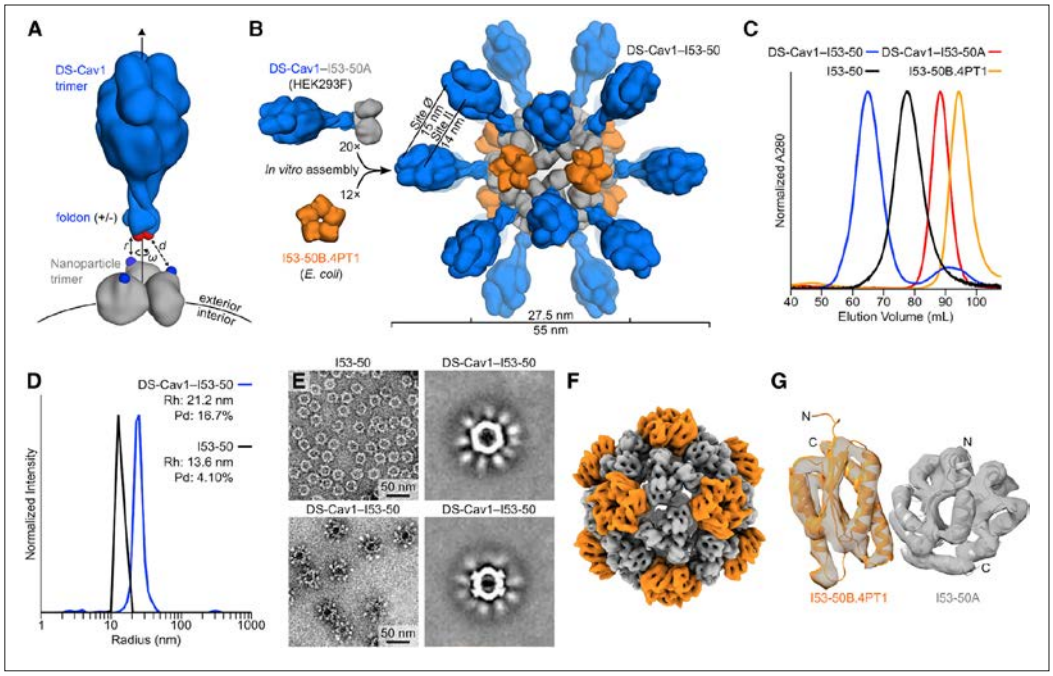

Figure 2.
Design, in vitro Assembly and Structural Characterization of DS-Cav1-I53-50.
Schematic representation of the computational docking protocol with biophysical and structural characterization of the nanoparticles.

HCMV Envelope Glycoprotein Diversity.
Mathilde Foglierini, Jessica Marcandalli and Laurent Perez
Human cytomegalovirus (HCMV) is the viral leading cause of congenital birth defects and is responsible for important morbidity and mortality in immunosuppressed individuals. Considerable efforts were deployed in the last decade to develop a vaccine capable of preventing HCMV infection. However, in recent clinical trials, vaccines showed at best modest efficacy in preventing infection. These findings were in part explained by the high level of sequence polymorphism at the genomic level. We investigated if genomic variation also leads to antigenic variation, and performed a bioinformatics sequence analysis to measure the percentage of conservation at the amino acid level of all the proteins present in the virion envelope. We observed that antigenic variation is in large part limited to only three proteins and demonstrated that the two leading vaccine candidates are well conserved at the amino acid level. These results suggest that despite genomic polymorphism, antigenic variability is not involved in the modest efficacy observed in the recent clinical trials for an HCMV vaccine.

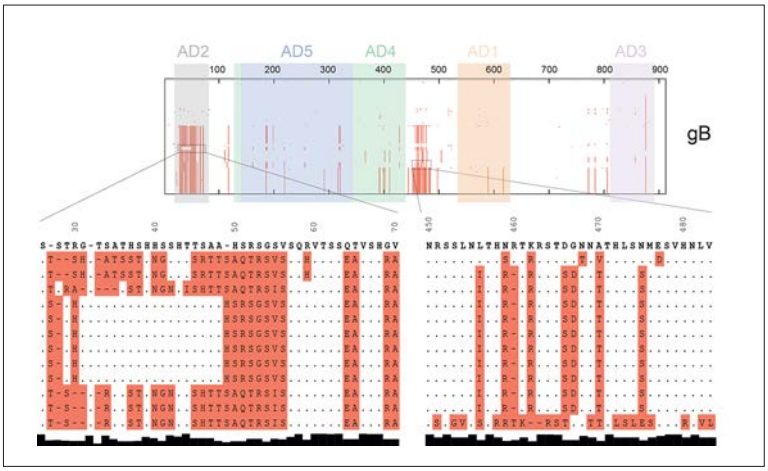

Figure 3.
Sequence alignment of the HCMV gB complex.
Protein sequences aligned with Clustal Ω. All amino acids different from the reference HCMV strain (Merlin) are highlighted in red.

Structure Based Nanoparticle Vaccine Design.
Michela Perotti, Jessica Marcandalli, Federica Sallusto and Laurent Perez
Our RSV nanoparticle results motivated us to continue the development of this promising nanoparticle platform for structure-based vaccine design. We are currently combining immune repertoire interrogation, computational design, structural biology and mice immunization to generate the next generation vaccine against different pathogens with no vaccine available. We are also investigating the potential of co-display to generate a broad immune response against pathogens belonging to the same family. Moreover, we are also exploring the potential advantages of antigen and immune activator co-display for nanoparticle self-adjuvantation.

Collaborators: NP King

Funding

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Collaborations

Claudio Ciferri, Genentech, Department of Structural Biology. South San Francisco, (US)

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Alexander Leitner, ETH Zürich, Institute of Molecular Systems Biology. Zürich, (CH).

Karin Loré, Karolinska Institutet, Department of Medicine Solna, Center for Molecular Medicine, Stockholm, (SE).

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A public antibody lineage that potently inhibits malaria infection through dual binding to the circumsporozoite protein.
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The IRB has state-of-the-art imaging facility (Flow cytometry and microscopy) headed by David Jarrossay who obtained a PhD degree at the University of Fribourg (CH). This facility is central to most of the research projects.
The 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.
Diego Morone, recruited in 2018, runs the microscopy facility. He is a microscopist and image analyst. He has experience in confocal microscopy, intravital microscopy and scientific image analysis. He teaches in the *Microscopy Course* organized by Prof. Thelen and aside offers a two-day internal course on *Image Analysis with ImageJ*.

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%). The lab also acquired a Sony SH800SFP cell sorter in a flow cabinet equipped with 4 lasers. Both sorters can perform cell disposition on plates.
- 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).
- 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 trouble-shooting. 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 offers training on instruments, 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 and MetaXpress (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 micro-scopes, comprising a surgical area for intra-vital microscopy:

- Confocal microscope.
  Leica TCS SP5, equipped with 4 high-sensitivity 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.
  The new *Molecular Devices ImageXpress Micro 4* – installed at the beginning of 2018 – is a widefield automated microscope for the acquisition and analysis of plates and slides. It provides 5 acquisition channels, an incubator for timelapse imaging and a robot for automated acquisition of up to 45 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 *FEI Live Acquisition* 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 BioTec 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).

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Fregno I. et al. *The EMBO Journal* 2018, e99259

Palomino-Segura M. et al. *JoVE* 2018, 138: e58355

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

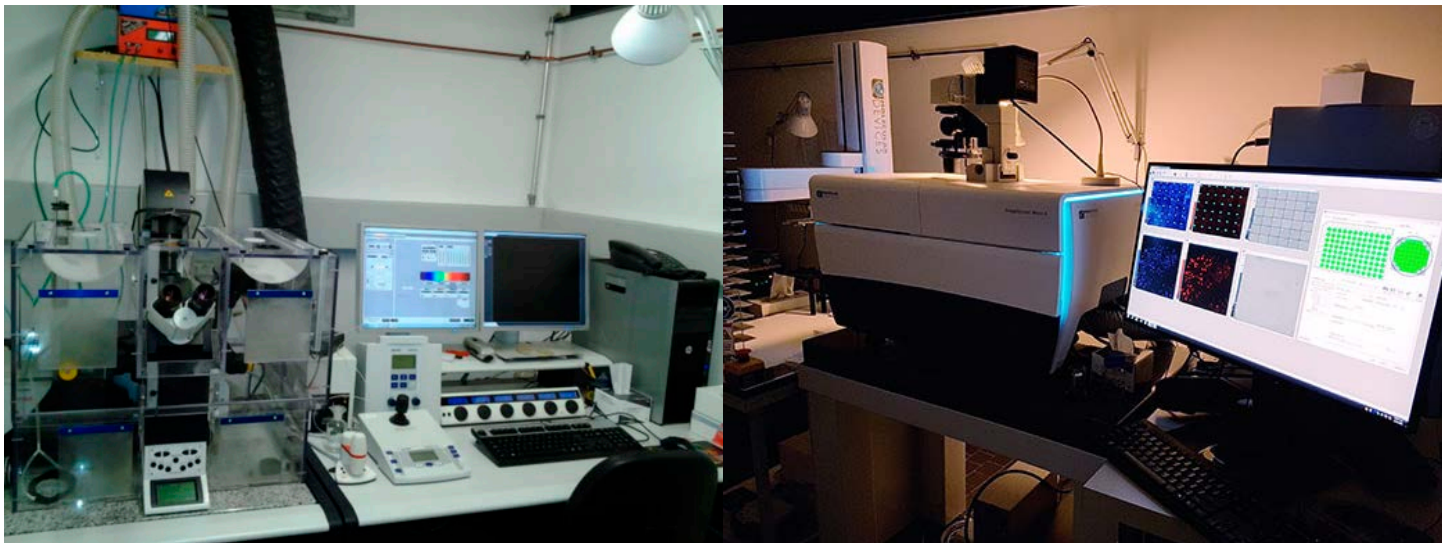

Figure 1.
Left: Confocal microscope Leica TCS SP5.
Right: High-content screening system Molecular Devices ImageXpress Micro 4.

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

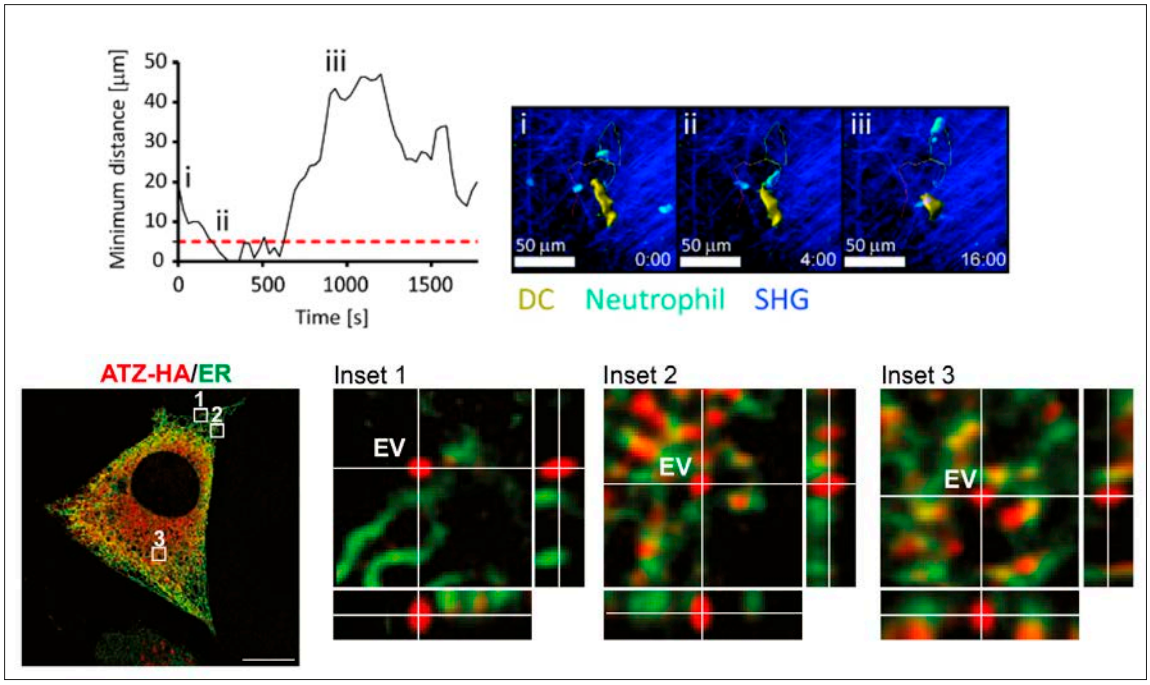

Figure 3.
Top: analysis of cell-cell contacts in intravital microscopy.
Bottom: resolution enhancement through 3D deconvolution allows the analysis of protein segregation in lysosomes.

NMR facility

The Nuclear Magnetic Resonance (NMR) facility provides access to a modern 600MHz Bruker Ascend spectrometer equipped with cryoprobe for increased sensitivity and helium recovery system for cost minimization.
The NMR facility main activity is the high-resolution structural investigation of protein and antibodies, but it is also suitable and available for small molecules, stability and quality control studies. The NMR is also equipped with an automatic sample loading robotic arm for metabolomics and similar studies.
Members of the facility are responsible for design and preparation of aptly isotopically labelled biological samples, data acquisition, processing and interpretation. Several publications in high impact journals such as *Cell* and *Nature Cell Biology* have already resulted from the facility work.

Bruker Avance 600 MHz
spectrometer with cryoprobe

Bioinformatics Facility

Overview

The Bioinformatics Core Unit supports the research groups with computational and statistical services. Importantly, more than just a supporting role, we proactively identify and develop novel bioinformatics projects that can complement and in many cases drive our biologic research. We develop innovative data analysis tools, visualization software and database resources for genomics research in collaboration with the Dalle Molle Institute of Artificial Intelligence (IDSIA).

Tasks

- Immune cell repertoire analysis.
- Analysis of next-generation sequencing data.
- Multi-omics data integration.
- Functional analysis at systems biology level.
- Statistical and computational support.

Equipment

Big memory compute servers: 64-core 512GB, 24-core 128GB + 2GPU.
Linux HPC cluster.

PhD programme


irb

The IRB PhD lecture course is supported by the Gustav & Ruth Jacob Foundation

PhD lecture course 2016 – 2017

Luca Guidotti
"Host-virus interactions in hepatitis B virus (HBV) infection: lessons from animal models"
San Raffaele Scientific Institute, Milan (IT) / 27.10.2016

Ido Amit
"The power of one: immunology in the age of single cell genomics"
Weizmann Institute of Science Rehovot, Israel (IL) / 15.12.2016

Peter D. Crompton
"Unraveling the cellular and molecular basis of immunity to malaria"
National Institute of Allergy and Infectious Diseases (NIAID), Rockville, Maryland (US) / 12.01.2017

Rudolf Aebersold
"Genotypic variation and the quantitative proteotype"
Institute of Molecular Systems Biology, ETH Zürich, Zürich (CH) / 08.02.2017

Sussan Nourshargh
"Neutrophil trafficking in vivo: modes, mechanisms and novel roles of chemokines"
William Harvey Research Institute, Barts and The London School of Medicine & Dentistry, Queen Mary, University of London, London (UK) / 09.03.2017

Maria Grazia Spillantini
"Protein aggregation in Alzheimer's and Parkinson's diseases: untangling the tangled brain"
University of Cambridge, Cambridge (UK) / 20.04.2017

Erika Pearce
"Mitochondrial shape shifting in the T cell response"
Max Planck Institute of Immunobiology and Epigenetics Freiburg (DE) / 18.05.2017

Gabriel D. Victora
"Clonal dynamics in antibody evolution"
The Rockefeller University, New York (US) / 08.06.2017

Stephen Kowalczykowski
"Understanding biology, one molecule at a time"
University of California, Davis, Davis, California (US) / 28.06.2017

PhD lecture course 2017 – 2018

Bodo Grimbacher
"Monogenetic causes for antibody deficiencies: How inflammation impairs specific antibody production"
Center for Chronic Immunodeficiency, University of Freiburg, Freiburg (DE) / 11.10.2017

Antonella De Matteis
"Regulation and function of the ER-Golgi contact site"
Telethon Institute of Genetics and Medicine (TIGEM), University of Napoli Federico II, Pozzuoli (IT) / 17.11.2017

Alexander Flügel
"The leptomeninges: a checkpoint for autoimmune effector T cells on their way into the CNS"
University Medical Center Göttingen, Göttingen (DE) / 06.12.2017

Ronen Alon
"Chemotactic cues and mechanical barriers for transendothelial migration of leukocytes and tumor cells"
Weizmann Institute of Science, Rehovot (IL) / 18.01.2018

Martin Jinek
"CRISPR-Cas genome editors: from mechanisms to applications"
University of Zürich, Zürich (CH) / 15.02.2018

Stuart Tangye
"How much is enough? Hyperactive PI3K signaling and immune dysregulation"
Garvan Institute of Medical Research, Sydney (AU) / 14.03.2018

Lorraine Symington
"Role of the Mre11-Rad50-Xrs2 complex in the DNA damage response"
Columbia University, New York (US) / 18.04.2018

Clare Lloyd
"Immune regulation in the airways"
Imperial College London, London (UK) / 07.06.2018

Seminar Programme 2017

Alan Tyndall
"Hematopoietic Stem Cell Transplantation in Autoimmune Disease- early success and future directions"
Department of Rheumatology, University Hospital of Basel, Basel (CH) / 30.01.2017

Andrew deMello
"High-Throughput Experimentation Drop-by-Drop"
Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich (CH) / 31.01.2017

Melania Balzarolo
"Molecular mechanisms underlying the pathogenic function of IL-23 in autoimmune neuroinflammation"
Institute of Experimental Immunology, University of Zürich, Zürich (CH) / 07.02.2017

Victor Greiff
Deciphering the rules of antibody repertoire complexity using a systems biology approach
Department of Biosystems Science and Engineering, ETH Zurich, Basel (CH) / 10.02.2017

Carl June
"CARs and Synthetic Biology"
Perelman School of Medicine, University of Pennsylvania, Philadelphia (US) / 15.03.2017

Matthias Mann
"Mass spectrometry-based proteomics and its application in biomedicine"
Max Planck Institute of Biochemistry, Martinsried (DE) / 11.04.2017

Hilde Cheroutre
"An Unpredicted Role for Retinoic Acid Receptor Alpha (RARα) in T cell Activation"
Division of Developmental Immunology, La Jolla Institute for Allergy and Immunology, La Jolla (US) / 21.04.2017

Mitchell Kronenberg
"Intestinal homeostasis mediated by a member of the TNF receptor super family"
La Jolla Institute for Allergy and Immunology, La Jolla (US) / 21.04.2017

Dmitriy Chudakov
"Profiling TCR and IG repertoires"
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow (RU) / 12.05.2017

Adriano Marchese
"Mechanisms governing endocytic trafficking and signaling of the chemokine receptor CXCR4"
Department of Biochemistry, Medical College of Wisconsin, Milwaukee (US) / 23.05.2017

Torsten Ochsenreiter
"Scientific Integrity"
Institute of Cell Biology, University of Bern, Bern (CH) / 19.06.2017

Teodoro Pulvirenti
"Scientific Publishing: What, How and Why?"
The Rockefeller University Press, New York (US) / 07.07.2017

Marinos Kallikourdis
"Integrating the unexpected roles of T cells in cardiovascular and oncological pathology"
Humanitas University, Milan, Italy & Humanitas Clinical and Research Center, Milan (IT) / 10.07.2017

Giuseppe Danilo Norata
"Translating adaptive immune cell's metabolism: a lesson from inherited and acquired dyslipidemias"
University of Milan, Milan (IT) / 12.07.2017

Kanika Vanshylla
"Membrane IgE intrinsic regulation of B cell antigen receptor signalling"
University Medical Center Göttingen, Göttingen (DE) / 12.07.2017

Leonie Brockmann
"Analysis of the molecular and functional heterogeneity of IL-10-producing CD4+ T cells"
University Medical Center Hamburg-Eppendorf, Hamburg (DE) / 18.07.2017

Anthony Jaworowski
"Persistent activation of innate immunity in virologically suppressed HIV+ individuals"
Burnet Institute, Melbourne, Victoria (AU) / 28.07.2017

Ping-Chih Ho
"You are fired up! Inflaming the tumor microenvironment"
University of Lausanne, Ludwig Center for Cancer Research, Epalinges (CH) / 29.09.2017

Enrico Lugli
"Discovering and rediscovering memory T cell differentiation, one cell at a time"
Humanitas Clinical and Research Center, Pieve Emanuele (IT) / 10.10.2017

Dan Itzhak
"Spatial proteomics: tracking intracellular protein movements without a microscope"
Max Planck Institute of Biochemistry, Martinsried (DE) / 03.11.2017

Sergio Lira
"Interplay of host microbiota, lymphocytes and CX3CR1+ cells in colitis"
Ichan School of Medicine at Mount Sinai, New York (US) / 07.11.2017

Francesco Marangoni
"Investigating autoreactivity by intravital microscopy: the case of tumor-associated T regulatory cells"
Massachusetts General Hospital and Harvard Medical School, Boston (US) / 19.12.2017

Seminar Programme 2018

Giorgio Colombo
"Protein dynamics and molecular design: computational approaches with an eye to chemical biology"
Università degli Studi di Pavia & Istituto di Chimica del Riconoscimento Molecolare, CNR, Milano (IT) / 08.02.2018

Dietmar Zehn
"Molecular control of T cell differentiation in acute and chronic infection"
School of Life Sciences Weihenstephan, Technical University of Munich (DE) / 13.02.2018

Herbert "Skip" Virgin
"Role of autophagy genes in immunity and inflammation"
Washington University School of Medicine, St. Louis and VIR Biotechnology, Inc., San Francisco (US) / 15.02.2018

Klaus Früh
"New Vaccine Strategies using cytomegalovirus"
School of Medicine, Oregon Health & Science University (US) / 19.02.2018

Isabel Poschke
"Composition and Dynamics of the Tumor Infiltrating Lymphocyte Repertoire in Patients with Pancreatic Cancer and Melanoma"
German Cancer Research Center (DKFZ), Heidelberg (DE) / 09.03.2018

Nicola Elvassore
"High-Efficiency Cellular Reprogramming and Differentiation in engineered micro-environment"
University of Padova and Venetian Institute of Molecular Medicine, Padova (IT) / 13.04.2018

Eric Gershwin
"Primary Biliary Cholangitis: Paradigm versus Paradox in Autoimmunity"
The University of California School of Medicine, Davis (US) / 19.04.2018

Aura Carreira
"Variants of unknown clinical significance, a genetic tool to interrogate the function of the breast cancer susceptibility protein BRCA2"
Homologous Recombination and Cancer, Curie Institute, Orsay (FR) / 26.04.2018

Sandra Schaffenrath
"Harnessing dendritic cells for cancer immunotherapy"
Department of Dermatology and Venerelogy, Medical University Innsbruck, Tyrol (AT) / 17.05.2018

Salvatore Valitutti
"On the fight between cytotoxic T lymphocytes and cancer cells at the immunological synapse"
Institut Universitaire du Cancer de Toulouse (IUCT), Toulouse (FR) / 22.05.2018

Deborah Burnett
"Antibody redemption through rapid mutations"
Garvan Institute of Medical Research, Darlinghurst, Sydney (AU) / 08.06.2018

Pietro Sormanni
"Rational design of antibodies against neurodegeneration"
Centre for Misfolding Diseases, University of Cambridge, Cambridge (UK) / 19.06.2018

Giampietro Corradin
"Malaria vaccine: discovery, development and clinical trials"
University of Lausanne, Department of Biochemistry, Epalinges (CH) / 05.07.2018

Silvio Bicciato
"Computational methods for studying the 3D genome architecture"
Center for Genome Research, University of Modena and Reggio Emilia, Modena (IT) / 31.07.2018

Gyorgy Snell
"Structural Biology of GPCRs"
Vir Biotechnology, Inc., San Francisco (US) / 08.08.2018

Jeffrey Liu
"In vivo brain GPCR signaling elucidated by phosphoproteomics"
Max Planck Institute of Biochemistry, Department of Proteomics and Signal Transduction, Martinsried (DE) / 09.08.2018

Thanos Halazonetis
"Oncogene-induced DNA replication stress in cancer"
Department of Molecular Biology, University of Geneva, Geneva (CH) / 19.09.2018

Valentina Proserpio
"Immunity, one cell at a time"
Italian Institute for Genomic Medicine (IIGM) and University of Turin, Turin (IT) / 15.10.2018

Matthew R. Hepworth
"Innate lymphoid cells (ILC) - critical regulators of mucosal barrier immune responses in health and disease"
The University of Manchester, Manchester (UK) / 23.10.2018

Burkhard Ludewig
"Redefining the nature of lymphoid tissue organizer cells"
Medical Research Center Kantonsspital St.Gallen, St. Gallen (CH) / 24.10.2018

Wolf-Dietrich Heyer
"Mechanisms of recombinational DNA repair: Dynamic reversal of pathway intermediates"
Department of Microbiology & Molecular Genetics, University of California, Davis (US) / 29.10.2018

Kai Xu
"Structural Biology Approaches to the Infectious Disease Research in AIDS, Malaria and Others"
Structural Biology Section, Vaccine Research Center, NIAID/NIH, Bethesda (US) / 30.10.2018

Marco Prunotto
"Identification of Target Antigens in Sera of Membranous Nephropathy Patients Using Whole Proteome Peptide Array Technology"
Roche and Genentech Late Stage Development, Hoffmann-La Roche AG, Basel (CH) / 21.11.2018

Alessandra Castegna
"Harnessing immunometabolism to fight cancer: opportunities from glutamine metabolism"
Biotechnologies and Biopharmaceutics University of Bari 'Aldo Moro', Bari (IT) / 14.12.2018

Triantafyllos Chavakis
"Immunometabolic crosstalk in innate immunity "
University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden (DE) / 18.12.2018

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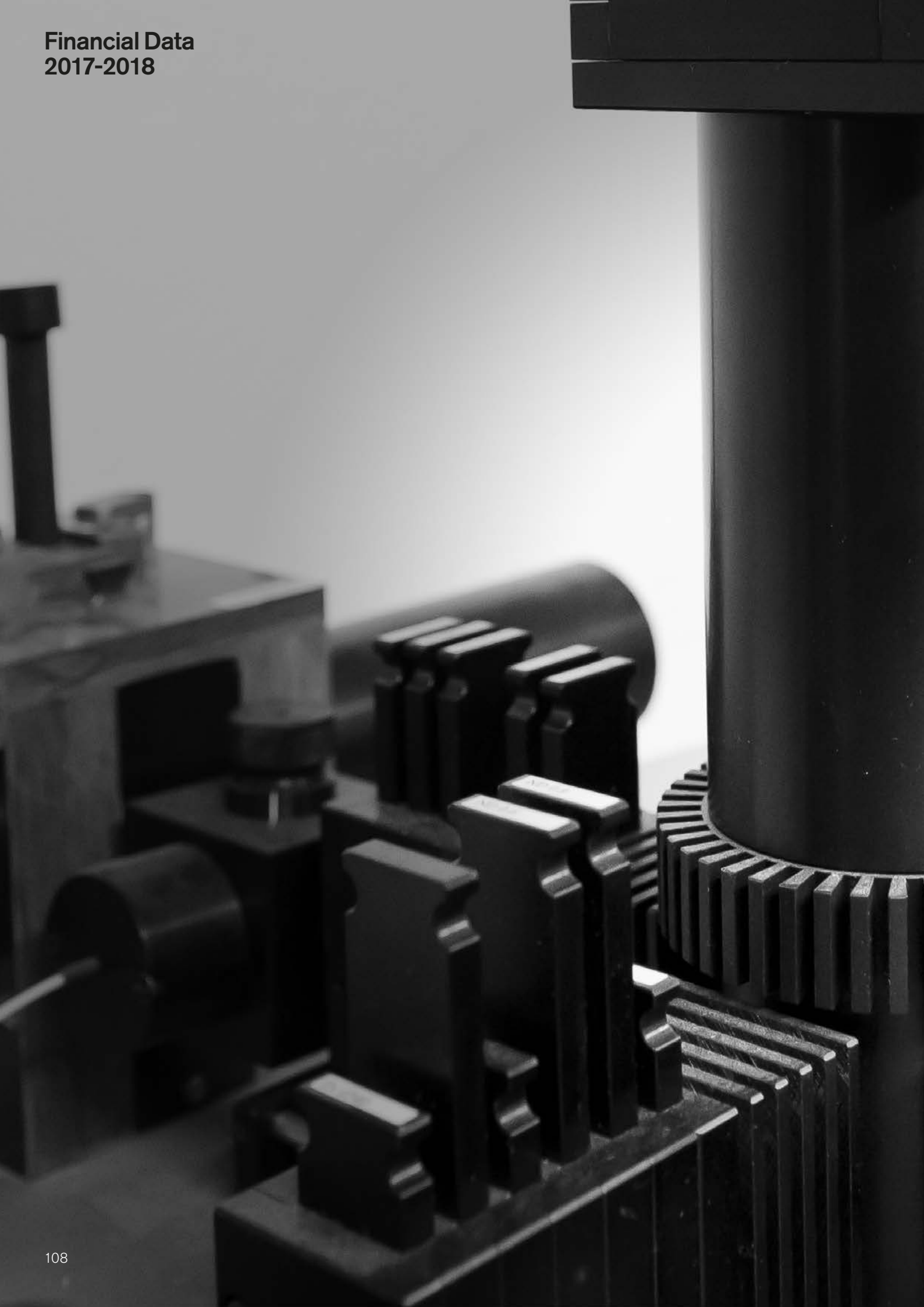
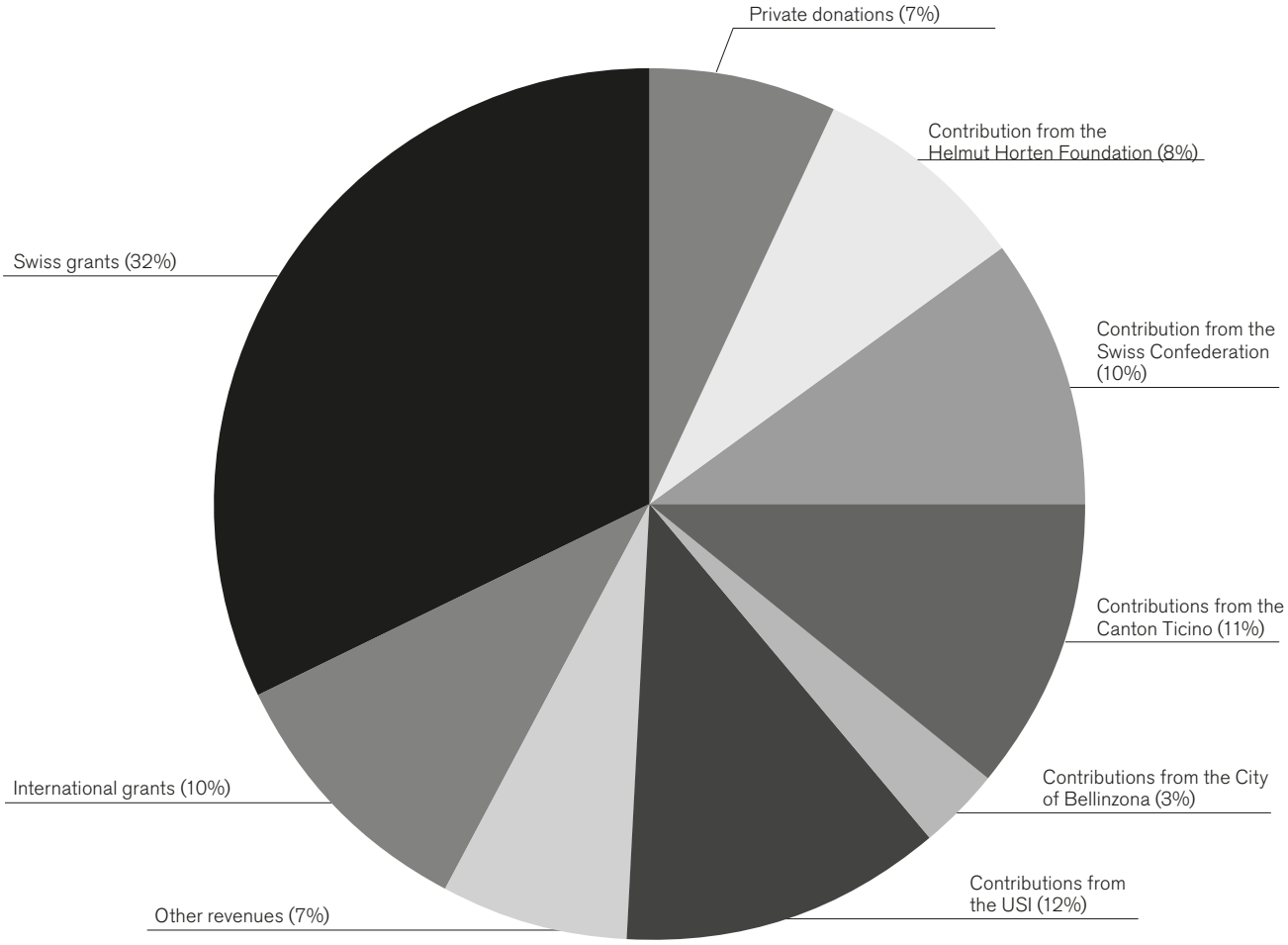
Financial Data 2017-2018



Financial Data 2018 (in Swiss Francs)

Funding by source 2018

After the arrival of three new research groups in the last 2 years, 2018 represented a year of consolidation of the research activities that increased reaching CHF 8.3 million, i.e., 42% of the entire budget of the IRB Foundation.
During 2018, the construction of the new IRB headquarters continued. The construction site should be completed by the beginning of 2021. The total investment amounts to approx. CHF 60 million, of which CHF 48 million for the building and CHF 12 million for its equipment and furnishings. One third of the building will be leased to the IOR (Institute of Oncology Research) and Laboratory for Biomedical Neurosciences (LBN) of the Neurocenter of Southern Switzerland (NSI).



Balance Sheet as of December 31, 2018 (In Swiss Francs)

Assets		31.12.2018	31.12.2017
1.	Liquidity	15'466'179	18'800'309
2.	Receivables	1'542'473	1'053'205
3.	Temporary receivables	3'614'598	3'890'128
	Current assets	**20'623'250**	**23'743'642**
4.	Participations	12'500	12'500
5.	Financial assets	10'844	10'824
6.	Buildings	11'298'883	5'366'398
7.	Other fixed assets	1	1
	Fixed Assets	**11'322'228**	**5'389'723**
	Total Assets	**31'945'478**	**29'133'365**

Liabilities		31.12.2018	31.12.2017
1.	Payables for goods and services	1'467'964	1'185'731
2.	Funds for Research Projects	5'356'763	4'465'081
3.	Funds for Laboratories	5'718'992	5'745'225
4.	Various Funds	1'000'262	1'102'973
5.	Accruals	432'992	357'910
	Current liabilities	**13'976'973**	**12'856'920**
6.	Fund for New Building	6'300'000	5'300'000
	Long term liabilities	**6'300'000**	**5'300'000**
7.	Capital resources	10'076'446	7'828'820
8.	Strategic Fund	1'500'000	3'100'000
9.	Annual Result	92'059	47'625
	Equity of the Foundation	**11'668'505**	**10'976'445**
	Total liabilities	**31'945'478**	**29'133'365**

Profit and Loss Account
for the year 2018
(In Swiss Francs)

Revenues		2018	2017
1.	Contributions from Swiss Confederation	1'925'000	1'994'700
2.	Contributions from USI	2'367'068	2'623'042
3.	Contributions from the Canton Ticino	2'188'285	2'188'285
4.	Contributions from the City of Bellinzona	622'280	632'240
5.	Contributions from the Helmut Horten Foundation	1'500'000	1'500'000
6.	Other Contributions	1'351'579	1'998'296
7.	Research Projects	8'309'428	8'044'613
8.	Overheads projects	732'595	456'512
9.	Other Revenues	705'345	558'609
	Total revenues	**19'701'580**	**19'996'297**

Costs		2018	2017
1.	Personnel Costs	10'309'755	9'677'784
2.	Consumables	2'206'756	2'259'500
3.	Rent and related costs	1'526'000	1'551'090
4.	Maintenance of buildings and equipments	800'287	846'646
5.	Investments	890'001	985'579
6.	Administrative Costs and Various	1'221'107	941'352
7.	Travels, Congresses and Guests	386'545	451'000
8.	Various Costs for Research	1'775'406	2'459'790
	Total operational costs	**19'115'857**	**19'172'741**
	Margin before depraciation, amortisation and non operational items	**585'723**	**823'556**
9.	Amortizations	0	431'148
	Operating result	**585'723**	**392'408**
10.	Fund increase	1'600'000	1'400'000
11.	Extraordinary contributions for New Building	-1'000'000	-1'000'000
12.	Total non operational and financial items and estraordinary costs	-106'336	-55'217
	Total non operational and financial items	**493'663**	**344'783**
	Annual result	**92'059**	**47'625**

Publications

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

Peer reviewed publications from January 2017

498.
Approaches to Detect microRNA Expression in T Cell Subsets and T Cell Differentiation.
Monticelli, S., T. Aijo and S. Trifari
Methods Mol Biol. 2017; 1514:153-172.

499.
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.

500.
The Mre11-Nbs1 Interface Is Essential for Viability and Tumor Suppression.
Kim, J. H., M. Grosbart, R. Anand, C. Wyman, P. Cejka and J. H. Petrini
Cell Rep. 2017; 18:496-507.

501.
Modulation of chemokine receptor function by cholesterol: new prospects for pharmacological intervention.
Legler, D. F., C. Matti, J. M. Laufer, B. D. Jakobs, V. Purvanov, E. Uetz-von Allmen and M. Thelen
Mol Pharmacol. 2017; 91:331-338.

502.
Dynamic intravital imaging of cell-cell interactions in the lymph node.
Stein, J. V. and S. F. Gonzalez
J Allergy Clin Immunol. 2017; 139:12-20.

503.
Role of SEC62 in ER maintenance: A link with ER stress tolerance in SEC62-overexpressing tumors?
Bergmann, T. J., F. Fumagalli, M. Loi and M. Molinari
Mol Cell Oncol. 2017; 4:e1264351.

504.
Dnmt3a restrains mast cell inflammatory responses.
Leoni, C., S. Montagner, A. Rinaldi, F. Bertoni, S. Polletti, C. Balestrieri and S. Monticelli
Proc Natl Acad Sci U S A. 2017; 114:E1490-E1499.

505.
The motor activity of DNA2 functions as an ssDNA translocase to promote DNA end resection.
Levikova, M., C. Pinto and P. Cejka
Genes Dev. 2017; 31:493-502.

506.
Social network architecture of human immune cells unveiled by quantitative proteomics.
Rieckmann, J. C., R. Geiger, D. Hornburg, T. Wolf, K. Kveler, D. Jarrossay, F. Sallusto, S. S. Shen-Orr, A. Lanzavecchia, M. Mann and F. Meissner
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