Scientific Report 2000 - 2005

Institute for Research in Biomedicine

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Institute for Research in Biomedicine

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President's Letter

The successful implementation of the Università della Svizzera Italiana (USI) led a group of local personalities active in medicine, economy and politics to become conscious of the necessity to promote basic biomedical research in Ticino. In June 1997 a Foundation was set up to create the Institute for Research in Biomedicine (IRB). The city of Bellinzona, the capital of the Canton of Ticino, was identified as the location and the Stabile Fabrizia, a 1964 steel and glass building by Architects Snozzi and Vacchini, was rapidly refurbished to host research laboratories.

The aim of the Foundation Council was to establish a center of excellence for biomedical research and higher education. To achieve this goal, the council selected as a director of IRB Professor Antonio Lanzavecchia, a world renowned scientist. In February 2000 the research activities started with the establishment of 6 independent groups led by competent and highly motivated scientists. Today the IRB counts 8 groups for a total of 52 collaborators (8 group leaders, 16 researchers, 16 students, and 12 technical and administrative personnel). The scientific progress and the teaching programme are evaluated by an international Scientific Advisory Board nominated by the Foundation Council and composed by leading scientists in the biomedical field.

Within a few years of activity, the IRB has gained an international recognition. This is documented by the numerous scientific publications, the success in obtaining extramural grants and in attracting young researchers and students from all over the world. In January 2003 the activities of IRB were assessed by the Centre for Science and Technology Studies (CEST), an independent institution for monitoring, evaluation and prospective activities serving as a basis for Switzerland's science and technology policy. The experts' evaluation acknowledged that the research conducted at IRB matches the criteria of international scientific excellence and recommended increased subsidize by the Swiss federal government. The IRB is well connected with other immunology institutions, universities and biotechnology and pharmaceutical companies. In Ticino, the IRB has established scientific collaborations with the Cantonal Microbiology Institute (CMI, which was relocated from Lugano to Bellinzona in 2003), the Oncology Institute of Southern Switzerland (IOSI, whose research laboratories are hosted in the IRB building since 2004), and the Cantonal Pathology Institute in Locarno. Collaboration agreements have been entered with the Università della Svizzera Italiana (USI), the Ente Ospedaliero Cantonale (EOC), the Centro Svizzero di Calcolo Scientifico (CSCS), and the Scuola Universitaria Professionale della Svizzera Italiana (SUPSI). The Foundation Council envisages a progressive integration of the IRB in the Swiss academic world in order to generate new synergies.

The IRB has increased its annual budget from 4 million Swiss francs in 2000 to about 9 millions in 2005. The research costs are covered primarily by competitive grants from funding agencies such as the Swiss National Science Foundation, the Swiss Cancer League, the European Union, and the US National Institute of Health. Infrastructural costs are covered by public contributions (the City of Bellinzona, the Canton of Ticino, the Swiss Confederation) and by private foundations and donations. The Helmut Horten Foundation has initially supported the IRB with a donation of 10 millions Swiss francs and continues to provide an essential annual contribution for running costs.

All members of the Foundation Council, stimulated by the outstanding results obtained in the first 5 years of activity, are increasingly committed to strengthen IRB and to secure its fruitful future within the Swiss scientific community.

Giorgio Noseda
President of the Foundation Council

Director's Letter

This report provides an overview of the first five years of activity of the Institute for Research in Biomedicine and describes the current research projects.

The mission of the Institute is to give an original contribution to biomedical research in the field of host defense from infectious agents, tumors and neurodegenerative diseases. Ten research groups have been operating in the first five years. The research activities range from transcriptional regulation and protein folding to receptor-ligand interaction and signaling, from leukocyte migration to regulation of the immune responses. The results are the subject of 130 publications, most of which in highly ranked journals. Particular emphasis is given to studies in the human system, since these may lead to a better understanding of pathophysiology and to novel therapeutic treatments.

Researchers at the Institute have reconstructed a functional human immune system in mice; have dissected chemokine networks and revealed new aspects of signal transduction and transcriptional regulation. Human memory T cell subsets with distinct function and migratory capacities have been identified as well as the signals involved in their generation and maintenance. The role of innate receptors on dendritic cells and B cells has been dissected and exploited. A method to isolate human monoclonal antibodies from memory B cells has been developed and the expression of antibody fragments in cells has been shown to prevent generation of harmful peptides.

The Institute plays a role in education by training graduate students through collaborations with Swiss Universities, in particular Basel, Bern, Fribourg, Lausanne, Zurich and participates in an international PhD programme with the Vita-Salute San Raffaele University in Milan, Italy. At present 16 students are enrolled and 11 have completed their training.

After 5 years of activity I am confident that the IRB has fulfilled the initial expectations and has become an internationally visible centre for basic and translational research. This fact is witnessed by the success of our researchers in attracting competitive grants from the Swiss National Science Foundation, the European Union, and other national and international bodies.

The Institute is especially fortunate in receiving core funding from its main sponsors, the Helmut Horten Foundation, the Cantone Ticino, the Swiss Confederation and the city of Bellinzona. Our gratitude also goes to the many individuals who support us through gifts and fellowships. We hope that the progress and achievements of the Institute will reward their dedication to the advancement of science.

Antonio Lanzavecchia, MD
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Protein Folding and Quality Control

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Introduction

The endoplasmic reticulum (ER) is site of synthesis, folding and quality control for secretory proteins, for proteins that operate at the plasma membrane and in intracellular organels of the endo-/exocytic pathway. As a rule, only native and fully assembled proteins leave the ER to be transported at their final destination, whilst defective polypeptides are destroyed. The aim of our work is to understand how ER-resident molecular chaperones and folding enzymes assist maturation of newly synthesized polypeptides, how the ER quality control machinery operates to prevent export of folding-defective polypeptides from the ER lumen and how the ER-resident chaperones regulate dislocation of misfolded polypeptides from the ER lumen into the cytosol for proteasome-mediated destruction.

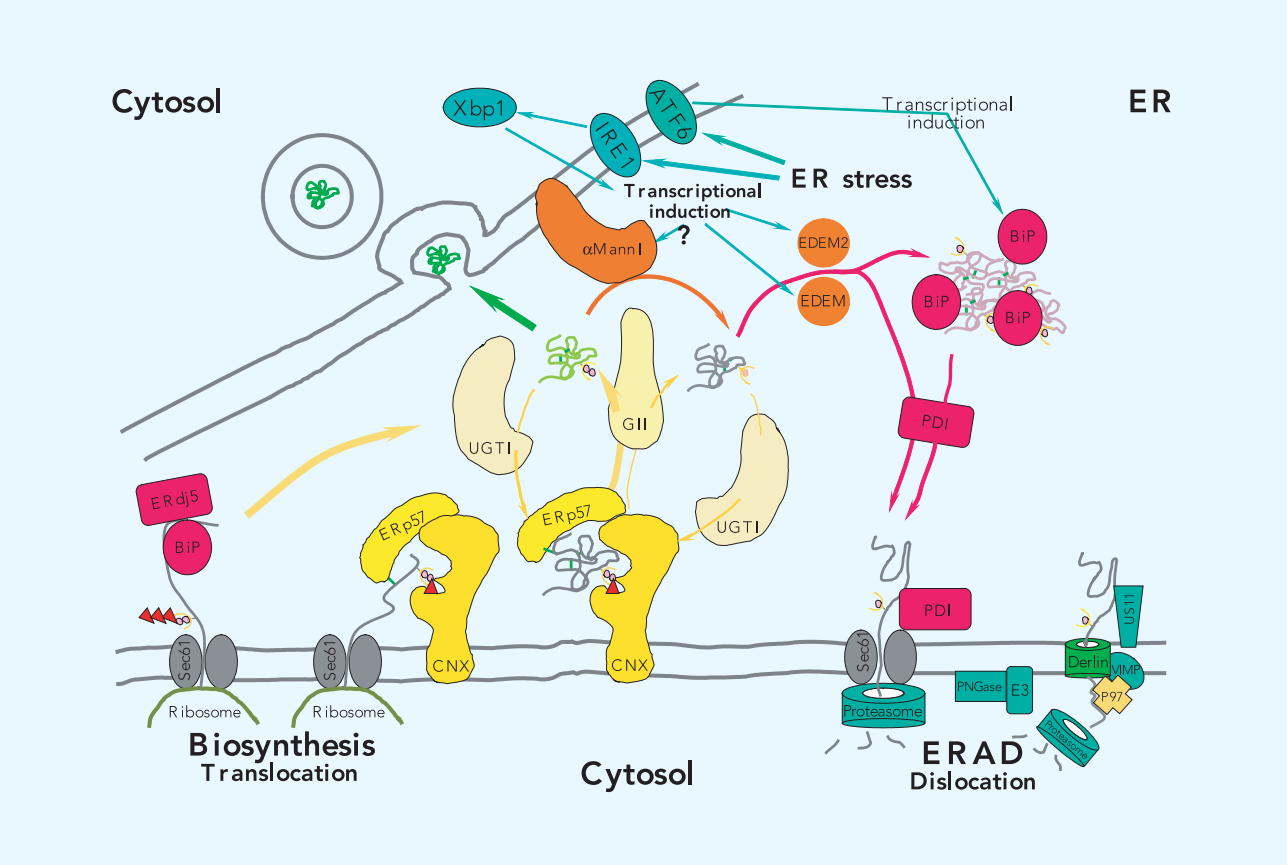
Protein folding in the ER

The majority of the proteins, which traverse the secretory pathway, receive Asn-linked glycosylations. These bulky hydrophilic modifications serve a variety of structural and functional roles within the cell, e.g., they increase solubility of folding intermediates and mediate association of newly synthesized polypeptides with the ER-resident molecular chaperones calnexin and calreticulin (Hebert et al., 2005). Comparison of protein folding in cells with and without calnexin or calreticulin showed that transient association of newly synthesized glycoproteins with the two lectin chaperones decreased the folding rate, thus making folding more efficient. Calnexin- or calreticulin-depletion poorly affected folding of most cellular and viral proteins; a dramatic loss of stringency in the ER quality control with transport at the cell surface of misfolded glycoprotein conformers was only observed when substrate access to both calreticulin and calnexin was prevented. As often postulated but never demonstrated, we showed that calreticulin and calnexin serve in combination, but also individually, as key retention factors for misfolded conformers, thus assuring fidelity of glycoprotein expression (Molinari et al., 2004).

ER-associated protein degradation

Most of our work is aimed at understanding how cells eliminate aberrant polypeptides generated in the ER by dislocating them into the cytosol for proteasome-mediated disposal in processes collectively defined as ER-associated degradation (ERAD). These processes are exploited by human pathogens such as bacteria and viruses to invade our cells or to escape immunosurveillance and their mis-functioning causes several human *conformational diseases* (such as cystic fibrosis, α1-antitrypsin deficiency, neurodegenerative diseases caused by protein aggregation).

Investigating the intracellular fate of several folding-defective variants of the human beta secretase (BACE) we contributed to the knowledge of mechanisms devised by cells for driving misfolded proteins from the ER lumen for degradation. In particular, we unravelled the role of calnexin-association in protecting non-native polypeptides from premature degradation and of EDEM as the ER-resident factor that regulates interruption of futile folding-attempts to promote ERAD of terminally misfolded polypeptides. We identified three variants of EDEM in the human and mouse genome and characterized their involvement in the ERAD process. We also showed that their intracellular level is increased upon ER stress and we identified in Xbp1 the transcription factor regulating EDEM variants expression under



Protein folding and quality control in the mammalian endoplasmic reticulum

stress conditions. Our findings that cells with impaired regulation of the EDEM level were also characterized by sub-optimal protein folding highlighted the importance of an efficient ERAD machinery to optimally accomplish protein folding and quality control. Based on our data, we proposed splitting of the ERAD process in 4 phases.

1. Folding-defective glycopolypeptides are at first subjected to unproductive folding attempts in the calnexin cycle. During this phase, ER-resident alpha mannosidases sequentially cleave mannose residues from N-linked glycans generating a degradation signal.
2. EDEM variants associate with de-mannosylated misfolded polypeptides promoting their transfer from the calnexin cycle into the BiP/PDI chaperone system.
3. BiP and PDI operate the polypeptide-unfolding which is required for dislocation into the cytosol for
4. Proteasome-mediated destruction (Eriksson et al., 2004; Molinari et al., 2003; Molinari et al., 2002; Olivari et al., 2005).

A novel approach to reduce cellular production of the toxic Aβ peptide

The human amyloid precursor protein (APP) is among the model-substrates used in our lab to investigate protein folding and quality control. Availability of a very specific monoclonal antibody used for immunoisolation of APP from cell lysates led use to the idea of setting-up an approach to inhibit generation of the toxic amyloid-beta (Aβ), a metabolite of APP cleavage operated by the cellular beta- and gamma-secretases. We mapped the monoclonal antibody epitope in the human APP molecule to find that it was located between residues 3 and 6 of the Aβ peptide. We prepared a vector for expression in mammalian cells of a single chain antibody (intrabody) formed by the variable heavy and light chain regions of the original monoclonal antibody covalently associated through a 15 aminoacids linker. We hypothesized that expression of this intrabody in the ER (and of a second variant of the same intrabody carrying an ER retention sequence) would result in association of the intrabody with newly synthesized APP, shielding of the APP's beta-secretase cleavage site adjacent to the intrabody epitope and consequent inhibition of Ab production. The experimental data confirmed our thoughts. One of our intrabodies associated with APP in the ER and followed the APP molecule throughout the secretory line strongly inhibiting beta-secretase-operated cleavage. The intrabody displaying the ER retention sequence also associated, and retained in the ER, newly synthesized APP preventing Aβ production (Paganetti et al., 2005).

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Paganetti P., Calanca V., Galli C., Stefani M., and Molinari M. beta-site specific intrabodies to decrease and prevent generation of Alzheimer's Abeta peptide. *J Cell Biol* 2005; 168:863-868

Ongoing projects

Protein aggregation as an intermediate step in ERAD

Silvia Olivari and Maurizio Molinari

Proteins that are unable to fold correctly in the ER are dislocated into the cytosol and degraded by the proteasome. The series of events eventually leading to ERAD may vary depending on the characteristic of the ERAD substrate and of the cell line. Previous work has shown that at the end of a lag phase consisting in unproductive folding attempts in the Cnx cycle, ERAD candidates are released from Cnx and enter, transiently, in BiP- and PDI-associated disulfide-bonded complexes before dislocation into the cytosol (Molinari et al. 2002). We also found that the intralumenal level of EDEM determines kinetics of ERAD by regulating glycoprotein release from the Cnx cycle (Molinari et al. 2003). The aim of this project is to investigate how EDEM expression in HEK cells affects the formation of disulfide-bonded aggregates.

The role of UDP-glucose:glycoprotein glucosyltransferase (GT) in glycoprotein quality control

Carmela Galli, Omar Vanoni and Maurizio Molinari

Newly synthesized polypeptides are co-translationally N-glycosylated by addition of pre-assembled, tri-glucosylated oligosaccharides on asparagines residues in Asn-X-Ser/Thr sequons. Terminal glucoses of N-glycans are rapidly trimmed by sequential action of glucosidase I and II. The product of these trimming reactions is a mono-glucosylated, protein-bound N-glycan that mediates association of the nascent polypeptide with the ER-lectins Cnx and Crt and the glycoprotein-specific oxidoreductase ERp57. Cleavage of the last glucose releases the glycopolypeptide from Cnx/Crt and exposes it to tight quality control operated by the GT. This enzyme specifically adds back one glucose on N-glycans of non-native proteins that require longer retention in the Cnx/Crt cycle. ER quality control makes sure that only native polypeptides leave the ER to be transported along the secretory pathway to their final destination. We are investigating consequences of depletion of the ER folding sensor GT on protein biosynthesis with the aim of better understanding the molecular mechanisms regulating protein quality control in the mammalian ER.

The role of the ER-resident oxidoreductase ERp57 in oxidative glycoprotein folding

Tatiana Soldà and Maurizio Molinari

The ER contains several molecular chaperones and folding factors that facilitate the folding and the assembly of newly synthesized polypeptides. The two lectin chaperones Cnx and Crt are associated with ERp57, a lumenal member of the protein disulfide isomerase (PDI) superfamily. ERp57 specifically promotes the oxidative folding of newly synthesized glycoproteins. The aim of this work is to determine consequences of ERp57 down-regulation and deletion on glycoprotein folding and to analyze if other ER resident oxidoreductases can replace ERp57. Cells showing substantial down-regulation of ERp57 have been obtained by RNA interference, upon intracellular expression of an ERp57 specific double-strand RNA formed by the sense an the antisense oligonucleotides connected by a short loop. ERp57 deletion is embryonic lethal for mice. To generate a ERp57-deficient cell line, fibroblasts were first prepared from ERp57$^{flox/flox}$ mice, which express wild-type levels of ERp57 protein. A cloned line immortalised with SV40 large T antigen was then transfected with a plasmid coding for cre recombinase to obtain stable ERp57 knockout fibroblasts ERp57 knockout cells. These cells will now be used to monitor oxidative folding of model glycoproteins such as influenza hemagglutinin and beta secretase.

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Transcriptional Regulation in Inflammation

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Introduction

The inflammatory and immune responses to infection rely on the coordinated activation and silencing of numerous, differentially expressed genes. Pivotal players in transcriptional regulation of several of them are the homo- and hetero-dimers generated by the five transcription factors of the NF-kB/Rel family.

The NF-kB family of transcription factors

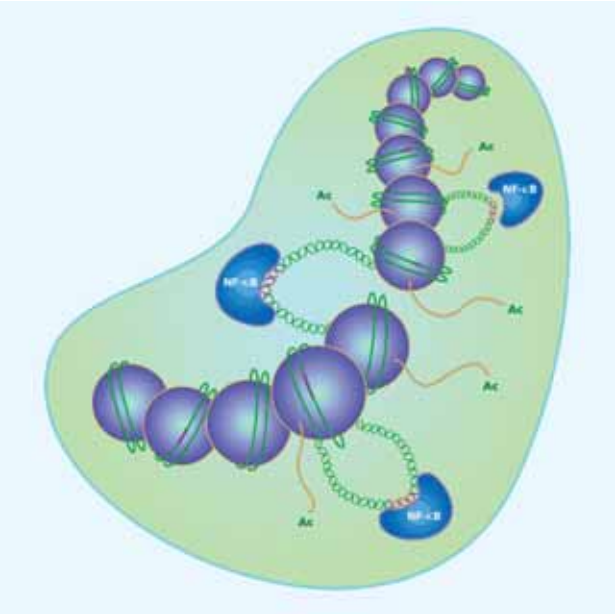
In mammals most cell types contain a collection of NF-kB dimers composed by homo- and hetero-typic combinations of the five transcription factors of the mammalian NF-kB/Rel family: p65/RelA, c-Rel, RelB, p50 and p52. These proteins contain a highly homologous Rel Homology Region (RHR) that mediates protein-DNA interactions and dimerization, as well as interactions with inhibitory proteins known as IkBs. Depending on the cell type and the differentiation status, the relative abundance of each dimer may vary, thus generating a high degree of complexity. The main regulatory switch in the NF-kB system is cytoplasmic and consists in the release of NF-kB from the IkBs. This activation step is mediated by the recently discovered IkB kinase (IKK), which phosphorylates amino-terminal regulatory serines in the IkBs and targets them for proteasomal degradation, thus liberating the NF-kBs and allowing them to enter the nucleus. However, it is becoming increasingly clear that in addition to this required activation step, both NF-kB recruitment to target genes and post-recruitment NF-kB-induced transcriptional events are actively regulated. All NF-kB dimers share the ability to bind a family of 9-11 nt DNA-binding sites collectively known as kB sites and conventionally represented as $G_{-5}G_{-4}G_{-3}R_{-2}N_{-1}N_0Y_{+1}Y_{+2}C_{+3}C_{+4}$ (R=purine, N= any nucleotide, Y= pyrimidine). In spite of similar DNA-binding profiles, NF-kB dimers are not equivalent in terms of transcriptional activation properties since each one of them activates specific subsets of genes and has different potency at genes that are activated in a redundant fashion.

Nuclear regulation of NF-kB activity

The molecular mechanisms leading to the activation of NF-kB in response to inflammatory agonists have been clarified in great detail in the last years: the major efforts by most labs were aimed at understanding the cytoplasmic events leading to NF-kB activation (i.e. release from the cytoplasmic inhibitors, the IkBs) and allowed to identify the IkB kinase (IKK) complex as the central and critical signal transducer of the pathway. The major focus of our lab has been to analyze (mainly in primary human cells of the monocytic lineage, namely dendritic cells and macrophages) mechanistic aspects of the nuclear regulation of NF-kB function. In particular, we have been interested in understanding how NF-kB is recruited to target genes, how specific genes depend on specific NF-kB dimers for transcriptional activation, and how kinetics of activation and switch off of individual genes are regulated. It is an obvious observation that although hundreds of genes contain NF-kB-responsive elements (kB sites) in their regulatory regions, their expression pattern can significantly vary from both a kinetic and quantitative point of view, and cell-type-specific patterns of expression are commonly found. Highly attractive features of this model of gene regulation are represented by 1) the *kinetic complexity* of gene induction (asynchronous activation and shut-off of different NF-kB-dependent genes), which may underlie different requirements for specific chromatin modifications/partner transcription factors/ co-activators in the induction of specific genes; 2) the existence of *multiple family members* exerting, in a cell-type specific fashion, redundant effects at some genes and divergent or opposite effects at others. We have found that recruitment of different NF-kB

dimers to target genes occurs in temporally distinct phases. Some genes recruit NF-kB dimers with fast kinetics, and usually no lag-phase between NF-kB nuclear entry and recruitment to these genes is observed. Several other genes recruit NF-kB with much slower kinetics in spite of the presence of high affinity sites in their promoters, and often after other TFs have been recruited to the gene and chromatin has undergone detectable modifications. Therefore, these data support the important concept that the kinetics of NF-kB recruitment to chromatin is regulated and that regulation is cell-type specific. We speculate that this temporal level of regulation is extremely relevant from a biological point of view because it allows the cell to induce the expression of each specific NF-kB dependent gene with kinetics that suit its specific function (rather than inducing all the NF-kB-activated genes at the same time). In addition, in a number of different situations NF-kB recruitment to specific targets does not occur in spite of NF-kB presence in the nucleus.

Our working model is that slow recruitment reflects a poor accessibility of the kB sites in some genes: other TFs must initially bind the gene to promote recruitment of enzymatic activities required for chromatin remodeling. While genes with immediately accessible sites represent a common pool of genes that are activated by most or all NF-kB inducers, genes that have to undergo remodelling to allow NF-kB recruitment have much higher requirements to be transcribed and as a consequence are induced only by a subset of stimuli capable of providing all the signal(s) required for activation. Adding complexity to this picture are two observations: first NF-kB recruitment to target genes is all but static and imaging data we are starting to collect suggest that the residency time of NF-kB on target genes in vivo may be extremely short, in a seconds time-scale; consistent with this dynamic view, we can frequently find that during the course of the response one type of NF-kB dimer is replaced by a different dimer whose transcriptional activity in the context of that specific gene is higher or lower than that of the initial dimer (thus laying the ground for a fine-tuning of the response over time). Second most NF-kB-dependent genes contain an unexpectedly high number of kB sites, as assessed by a refined computational method for the search of conserved sites in long genomic regions. This suggests that different sites scattered along the promoter or within the gene may nucleate different NF-kB-dependent promoter (or enhancer) complexes; we are testing this possibility as well as the idea that each site may serve a specific function in specific cell types or in response to specific agonists or during a specific phase of the response. In addition, the sequence of the kB sites (which are rather degenerated) exerts an allosteric effect on the bound NF-kB dimer, as clearly shown by resolution of crystals generated with different kB sites: this suggests that not only the presence of multiple kB sites but also their specific sequence may be of relevance to NF-kB regulation, a hypothesis we are directly testing.



NF-kB recruitment to target promoters
(Cover story from J Exp Med 2001; 193:1351).

A major long-term goal of our research is the identification of the transcriptional partners mediating the transcriptional effects of NF-kB. We have initially tackled this issue with two different strategies: first we are analyzing in detail the assembly of a number of model NF-kB-dependent promoters looking at known GTFs, mediator components, partner TFs in order to define and describe general behaviours occurring at NF-kB-dependent promoters. Second, we are analyzing how, and at which step, the absence of signal transducers or components of the transcriptional apparatus required for the induction of a subset of NF-kB-dependent genes influences the assembly of NF-kB-dependent promoter complexes.

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Ongoing projects

Regulation of the inflammatory transcription factor NF-kB in vivo

Simona Saccani, Daniela Bosisio, Ivan Marazzi, and Gioacchino Natoli

Nuclear Factor kappa B (NF-kB) is a family of transcription factors that are rapidly and transiently activated in response to most inflammatory stimuli and are required for transcriptional activation of several inflammatory and immune response genes. Aim of this project is to define the mechanisms regulating recruitment of NF-kB to target genes and post-recruitment NF-kB function. We have already shown that a chromatin-dependent regulatory mechanism generates two distinct classes of NF-kB-dependent genes: those containing constitutively and immediately accessible NF-kB sites and those that have to be conformationally modified to become accessible to NF-kB before the termination of the response. Remarkably, various NF-kB activators are different in their ability to make the latter genes accessible to NF-kB, which in turn depends on their ability to activate collateral signal transduction pathways like the p38 MAPK.

Dimers composed of different NF-kB proteins have a different transcriptional activity at target genes: exchange of dimers is exploited by the NF-kB system to finely tune transcriptional activity of different genes over time. Both a fast exchange between chromatin and nucleoplasmic compartment and proteasomal degradation of promoter-bound NF-kB contribute to catalyze an exchange of dimers. Detailed and mechanistic analysis of NF-kB regulation in cells lacking individual NF-kB proteins is ongoing and is clarifying how each NF-kB subunit contributes to the assembly of transcriptionally active promoters, to the recruitment of partner transcription factors and to the termination of the response.

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Signal Transduction

Laboratory

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Introduction

Leukocyte trafficking is largely controlled by chemokines and their respective receptors. Locally expressed chemokines recruit distinct leukocyte populations by interacting with specific receptors. The process occurs in normal and in pathological conditions. From a biochemical standpoint migration is an active process mediated by several signal transduction pathways and is accompanied by cell polarization, with a morphological distinct front and a rear end. Migration is ultimately powered by remodeling of the actin cytoskeleton at the leading edge and the sequential activation of adhesion molecules. The coupling of chemokine receptors to the downstream effector pathways which govern these activities is not well understood. Several lines of evidence indicate that chemokine receptors induce heterogeneous cellular responses and that this signaling divergence occurs in close proximity of the receptors (Thelen M., 2001).

Chemokine receptor-mediated signalling

Investigations of our group revealed that in primary human lymphocytes chemokines by binding to selective receptors induce distinct responses, as evidenced by the activation kinetics of protein kinase B and the MAP-kinase cascade. The finding indicates that chemokine mediated signal transduction is receptor-specific and in this scenario not cell type specific (Tilton B. et al., 2000). The reported prolonged activation of protein kinase B by the CXCL12 (SDF-1) CXCR4 interaction could be the molecular basis of the antiapoptotic activity of the receptor found in many cell types.

It is generally assumed that the chemokine receptor CXCR4 and its ligand CXCL12 form a unique pair. We investigated the signaling capacity of CXCR4 at different stages of B cell maturation. During lymphopoiesis, CXCR4 is continuously expressed on the surface of B cells and mediates retention of the cells in the bone marrow. As the cells become mature their signaling profile changes. PreB and proB cells migrate towards CXCL12 and mobilize intracellular calcium, whereas mature B cells do not show these responses, albeit the cells retain the capability to migrate in response to CXCL13 and CCL21. Chemotactic responsiveness of mature B cells to CXCL12 is regained when the cells differentiate into plasma cells. By contrast, stimulation of B cells with CXCL12 at all stages of development results in the activation of the MAP-kinase cascade and in rapid CXCR4 internalization. The pathways leading to ERK1/2 activation are different in preB and mature B cells. In either case, ERK1/2 activation is pertussis toxin sensitive, but inhibition of PI 3-kinase causes only in mature B cells an almost complete block of ERK1/2 activation. Our current findings show that during B cell lymphopoiesis CXCR4 alters its coupling to downstream signal transduction pathways and suggest that receptor activity may depend on accessory proteins (Palmesino et al., *submitted*). Regulation by specific accessory proteins is further supported by the observation that in patients suffering from the WHIM syndrome the activity of wild type CXCR4 is selectively deregulated (Balabanian K. et al., 2005).

Signalling by different ligand-induced receptor active states

Antagonism of chemokines on chemokine receptors constitutes a new regulatory principle in inflammation. CCL11 (eotaxin), an agonist for CCR3 and an attractant of eosinophils, basophils, and Th2 lymphocytes, was shown to act as antagonist for CCR2, which is widely ex-

pressed on leukocytes and is essential for inflammatory responses. We described a novel mechanism how chemokine receptor function can be arrested by endogenous ligands. CCL11 binding to CCR2 stimulates the MAP kinases ERK1/2 and activation of this pathway is indispensable for CCL11-mediated attenuation of CCR2 function. ERK is also activated by CCR2 agonists, e.g. CCL2 (MCP 1). However, the involved pathways are different, although in either case coupling of CCR2 to pertussis toxin sensitive heterotrimeric G-proteins is necessary. The results suggest that CCR2 could assume different activation states depending on the ligand it encounters and, with respect to actin polymerization and calcium mobilization, these activation states trigger agonistic and antagonistic responses. In this way CCL11 could cause an attenuation of proinflammatory responses mediated by full agonists of CCR2 (Ogilvie P. et al., 2004).

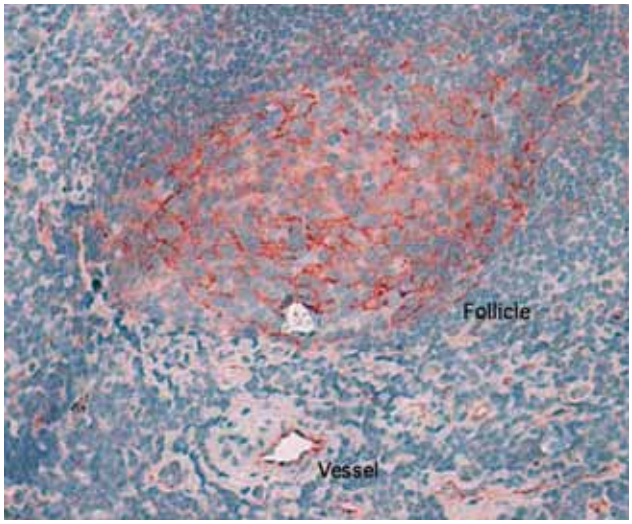
The notion that CCL11, as opposed to CCL2, does not induce actin polymerization suggests that coupling to the relevant pathway is not triggered by the specific chemokine-induced receptor active state. RhoGTPases are critical upstream elements in the regulation of actin polymerization. Activation of the GTPases is mediated by selective guanine nucleotide exchange factors (GEF). A candidate GEF in chemokine receptor-mediated signal transduction could be P-Rex1. This GEF is activated by βγ-subunits of heterotrimeric G-proteins and by products of PI 3-kinase. Our ongoing studies indicate that P-Rex1 undergoes transient posttranslational modifications that are induced by CCL2 but not by CCL11-mediated activation of CCR2.

Potential novel chemokine receptors

The orphan receptor RDC1 shows remarkable similarities to chemokine receptors and has been considered as a novel member of the family. However, no ligand for RDC1 has been found. PCR analysis of human peripheral blood leukocytes (PBL) performed in our laboratory revealed high levels of RDC1 mRNA in B cells and monocytes, moderate expression in $CD4^+$-T cells, basophils and NK cells.

The mRNA appears to be absent in $CD8^+$-T cells. ProB and preB cells express low levels of RDC1 whereas cells from more mature stages contain substantial amounts mRNA of RDC1. FACS analysis of PBL essentially confirmed and extended the PCR analysis indicating that RDC1 is expressed on B cells, monocytes, basophils and dendritic cells (DC). During maturation of mDC and pDC the expression of RDC1 appears to become more prominent.

FACS analysis of tonsil-derived leukocytes revealed that naïve and memory B cells express high levels of RDC1



RDC1 expression in tonsils

whereas centroblasts and centrocytes were moderately positive. Like in PBL also in tonsil-derived $CD4^+$-T cells RDC1 was found only in a subfraction. Immunohistochemistry (IHC) of tonsil sections reveals a prominent expression of RDC1 in B cell follicles. These results are consistent with the hypothesis that RDC1 could have some role in the differentiation of B cells (Infantino et al., *in preparation*). Ongoing attempts direct towards the identification of a specific ligand of RDC1.

PI 3-kinases

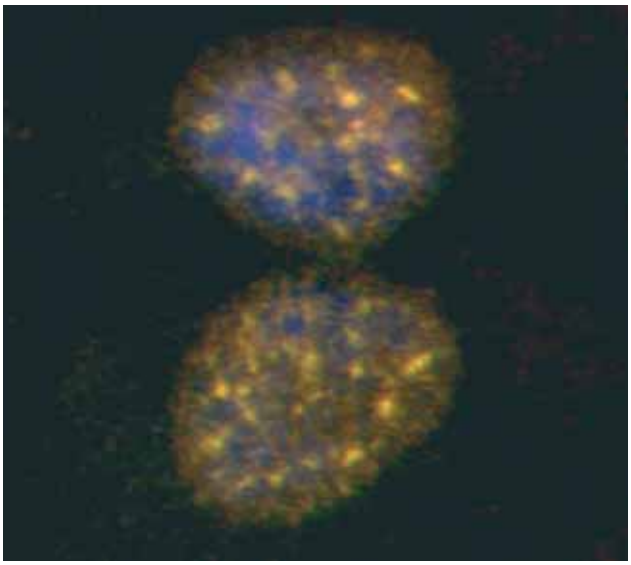
Phosphoinositide 3-kinases (PI 3 kinase) play important roles in signal transduction and the regulation of many cellular functions, including proliferation, viability, motility and vesicular transport. Their critical involvement in tumor formation, growth and spreading is generally accepted, but the underlying mechanisms are often not clear. Several isoforms are expressed in mammalian cells which fulfill distinct roles in cellular processes. Most research has focused on the class I enzymes which were first discovered. We have investigated the function of the human class II PI 3 kinase-C2α (HsPI3K-C2α). Our studies suggest an unexpected function of this enzyme in the regulation of cellular processes.

Subcellular localization studies demonstrate that HsPI3K-C2α has dual cellular localization being present in the cytoplasm and in the nucleus. A distinct nuclear localization signal (NLS) sequence was identified. The NLS was mapped to a stretch of 11 amino acids located within C2-like domain of the kinase. In the cytoplasm and the nucleus HsPI3K C2α associates with macromolecular complexes which are resistant to detergent extraction. Indirect immunofluorescence revealed that in the nucleus HsPI3K-C2α is enriched at distinct subnuclear domains, known as nuclear speckles, which contain

pre-mRNA processing factors and are functionally connected to RNA metabolism. Phosphorylation of HsPI3K C2α is induced by inhibition of RNA polymerase II-dependent transcription and coincides with enlargement and rounding up of the nuclear speckles (Didichenko S. A. et al., 2001).

We also investigated cell cycle dependent and genotoxic stress-induced phosphorylation of HsPI3K C2α. We observed that the kinase becomes phosphorylated upon exposure of cells to UV-irradiation and in proliferating cells at the G2/M transition of cell cycle. Stress dependent and mitotic phosphorylation of HsPIK3-C2α occurs on the same serine residue (Ser259) within a recognition motif for proline-directed kinases. Mitotic phosphorylation of HsPIK3-C2α can be attributed to Cdc2 activity and stress-induced phosphorylation of HsPIK3-C2α is mediated by JNK/SAPK. Phosphorylation appears to be a prerequisite for proteasome-dependent degradation of HsPIK3-C2α and may therefore indirectly contribute to the regulation of the activity of the kinase Our findings suggest that HsPI3K-C2α is involved in regulation of cell cycle progression and could (Didichenko S. A. e.t al., 2003) potentially play a role in tumorigenesis. Recent studies show that overexpression of HsPI3K-C2α in mammalian cells results in centrosome multiplication, abnormal spindle assembly and multinucleation as a consequence of incomplete cell division.

Similar abnormalities are often seen in human tumors. Generally the molecular mechanisms of centrosome regulation leading to genomic instability are poorly understood. Our discovery that HsPI3K-C2α is a bona fide centrosome component provides first evidence that regulation of the centrosome cycle might involve lipid interactions (Didichenko et al., *in preparation*).



Localization of HsPI3K-C2α at nuclear specles

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Ongoing projects

CXCR4 associated proteins and their role in cell specific receptor function

Elena Palmesino and Marcus Thelen

Understanding chemokine receptor-mediated signaling in different cellular environments is the main focus of the project. Ample evidence from our laboratory and by others indicate that coupling of a given G protein coupled receptor to downstream signaling cascades must be regulated in close proximity to the receptor and may vary between cell types. As a model we investigate the signaling properties of the chemokine receptor CXCR4 which regulates trafficking of leukocytes and tissue cells and is involved in tumor metastasis. The receptor also mediates cell survival and is important in organogenesis. However, CXCR4-stimulated intracellular signaling depends on the cell system. To characterize receptor-associated proteins, that determine the fate of CXCR4-medited cell activation, we developed a solubilization protocol that does not affect the structural integrity of CXCR4, and in which solubilized CXCR4 retains is ability to bind CXCL12. Current investigations should lead to the identification of receptor associated proteins in different cellular systems. Analysis of the expression patterns of the proteins will provide clues on their function in the regulation of CXCR4 activity.

RDC1, an orphan receptor with similarities to chemokine receptors

Simona Infantino and Marcus Thelen

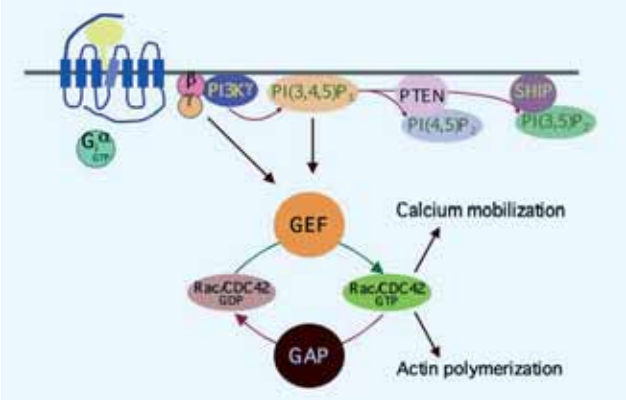
The orphan receptor RDC1 may function as a chemokine receptor. We have characterized its expression pattern in leukocytes, in particular on B cells. So far, we have tested most of the known human chemokines as potential ligands, but could not identify a specific agonist. Receptor internalization on primary and transfected cells was measured as agonist induced response. Recent results obtained in collaboration with our partners in Paris suggest that CXCL12 binds RDC1 and induces its internalization in T cells. However, results form our laboratory indicate that primary B cells do not respond to CXCL12, but to a yet unknown agonist. We assume that the physiological relevant ligand could be an unknown molecule. To this end supernatants derived from different cell culture systems, mimicking the environment where RDC1 positive cells reside, are tested for potential activity on RDC1 and will be fractionated using standard biochemical techniques. Once a ligand has been identified we will study its expression patter and physiological significance.

Role of the guanine nucleotide exchange factor P-Rex1 in chemokine receptor mediated signaling

Sylvia Thelen and Marcus Thelen

The small RhoGTPases induce actin polymerization and are therefore critical regulators of cell migration. It is assumed that the selective activation of RhoGTPases which are widely expressed occurs via specific guanine nucleotide exchange factors (GEF). Thus, the activation of a specific GEF mediates local chemokine receptor-dependent actin remodeling.

We have observed that the GEF P-Rex1 undergoes multiple transient posttranslational modifications that are induced upon binding of chemokines to their cognate receptors. The project aims in the identification and the functional characterization of these posttranslational modifications. It is expected that some of the modifications will influence the subcellular localization and activity of P-Rex1. Elucidation of potential pathways that lead to the activation of the GEF could reveal the coupling of chemokine receptors to actin polymerization.



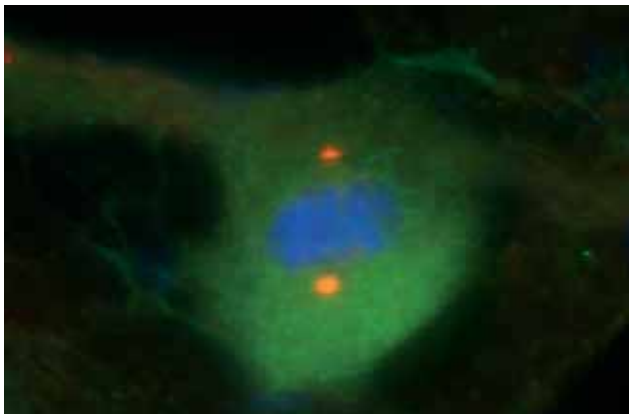
Model of chemokine receptor-dependent activation of Rho GTPases

Cellular function of HsPIK3-C2α

Marcus Thelen

The project is dedicated to the elucidation of the cellular function of HsPIK3-C2α in order to disclose the role of the kinase and its lipid products in cell cycle regulation and tumor formation.

The characterization of the spatiotemporal activity of HsPIK3-C2α will provide new insights into its function. Colocalization studies of the kinase with its potential lipid products by means of appropriate fluorescence markers in living cells will reveal «hot spots» where the activity of the kinase is critical for cellular homeostasis. Introduction of HsPIK3-C2α mutants into the cells as well as loss of function experiments employing small interference RNA technique shall corroborate involvement of HsPIK3-C2α in regulatory steps during the cell cycle and DNA repair. Identification of proteins that interact with HsPIK3-C2α in the nucleus and at the centrosome is relevant for the understanding of potential pathways.



Localization of HsPI3K-C2α at centrosomes

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Hematopoietic Development

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Laurie Chicha, PhD	2002-2005

Introduction

Throughout life, a small fraction of hematopoietic stem cells (HSCs) self-renew in the bone marrow and continuously generate all cells of the hemato-lymphoid system, a system with high cellular turn-over. Because of its ready accessibility, hematopoiesis is currently one of the best studied mammal adult stem cell differentiation systems, and is likely paradigmatic for other physiologic (e.g. liver, skin, central nervous system) and pathologic (tumors, leukemia) stem cell regenerated compartments. Beyond its model character for basic research, hematopoietic stem cell transplantation (formerly bone marrow transplantation) is the so far only successfully working clinical stem cell therapy, mostly applied for the treatment of malignant hematologic disease or immunodeficiencies. Also, hematopoietic stem cells currently provide the major gateway for clinical gene therapy.

The hierarchically structured, unidirectional differentiation process from HSCs to terminally differentiated cells involves progressive loss of self-renewal ability, proliferation capacity, and lineage differentiation potentials.

We are studying how this process is regulated, with a special focus on the development and function of antigen presenting cells (Figure 1). Our hypothesis is that once HSCs enter the maturation process, subsequent commitment and expansion to different cellular lineages is largely controlled externally, depending on varying demand. To evaluate this, we characterize and isolate human and murine hematopoietic progenitor cells at critical checkpoints of development, study and subsequently modify their transcriptional profile, and test their responsiveness to physiologic stimuli and to pharmacologic compounds in both *in vitro* and *in vivo* assays.

In depth understanding of physiologic differentiation pathways from HSCs to mature cells of the hematopoietic system will eventually provide new insights and improved therapeutic methods to treat hematopoietic and immune system diseases.

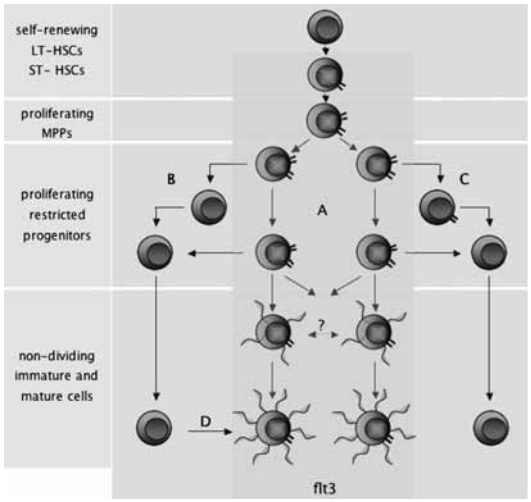

Figure 1. Proposed «Flt3-licence» working-model for steady-state dendritic and natural interferon producing cell development from early hematopoietic progenitor cells. (A) «Flt3-licence pathway» for DC and IPC development, (B) Flt3 down-regulation and loss of DC and IPC developmental options, (C) loss of DC and IPC developmental options in Flt3 positive cells due to competitive, dominant lineage-restriction signals, (D) non-steady-state, inflammatory, Flt3 independent DC developmental pathway.

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Ongoing projects

Human dendritic cell development

Laurie Chicha, David Jarrossay, and Markus Manz

Because of different cytokine responsiveness, surface receptor, and transcription factor expression, human $CD11c^-$ natural type I interferon–producing cells (IPCs) and $CD11c^+$ dendritic cells (DCs) were thought to derive through lymphoid and myeloid hematopoietic developmental pathways, respectively. To directly test this hypothesis, we used an *in vitro* mouse stroma cell based assay (Ac6) allowing simultaneous IPC, DC, and B cell development, and we tested human lymphoid and myeloid committed hematopoietic progenitor cells for their developmental capacity. Lymphoid and common myeloid and granulocyte/macrophage progenitors were capable of developing into both functional IPCs, expressing gene transcripts thought to be associated with lymphoid lineage development, and into dendritic cells. However, clonal progenitors for both populations were about fivefold more frequent within myeloid committed progenitor cells. Thus, in humans as in mice, natural IPC and dendritic cell development robustly segregates with myeloid differentiation. This would fit with natural interferon type I–producing cell and dendritic cell activity in innate immunity, the evolutionary older arm of the cellular immune system.

We are now characterizing the transcriptional profiles from HSCs to IPCs and DCs by isolation of differentiating cells at each cellular division. Furthermore, we try to modify this differentiation process via the addition of different cytokines and innate stimulators of the immune system. This will enable us to identify critical stepwise genetic events involved, and possibly will provide new approaches to influence the differentiation process.

Flt3-ligand/Flt3 tyrosin kinase regulation of mouse natural type I interferon-producing and dendritic cell development

Nobuyuki Onai, Aya Onai, Roxane Tussiwand, and Markus Manz

Flt3-ligand is a non-redundant cytokine in type-I interferon-producing cell (IPC) and dendritic cell (DC) development (as shown in Flt3-ligand deficient mice), and IPC and DC differentiation potential is confined to $Flt3^+$-hematopoietic progenitor cells. We currently study over-expression of human *Flt3* in $Flt3^-$-and $Flt3^+$-progenitors and found that Flt3 expression and stimulation rescues and enhances their IPC and DC differentiation potential, respectively. In $Flt3^-$ megakaryocyte/erythrocyte restricted progenitors (MEPs), enforced Flt3-signaling induces transcription of IPC-, DC-, and GM-development affiliated genes and activates differentiation capacities to these lineages. Moreover, ectopic expression of Flt3 downstream transcription factors STAT3 or PU.1 in $Flt3^-$ MEPs instructs differentiation into IPCs, DCs, and myelomonocytic cells. This might suggest an environmental Flt3-ligand cytokine driven model, where Flt3 acts as the earliest inducer and a constant enhancer of steady-state IPC and DC generation.

Dendritic cell developmental potential: new targets for immunomodulation

Roxane Tussiwand, Nobuyuki Onai, Markus Manz

Dendritic and natural interferon-producing cell progenitors and their downstream steady-state cell populations express the Flt3 receptor. Flt3-ligand$^{-/-}$ mice have massively reduced, and Flt3-ligand-injected mice develop markedly increased numbers of both cell types. Thus, *in*

vivo dendritic and natural interferon-producing cell development is largely dependent on Flt3 signaling. We therefore reasoned that pharmacologic inhibition of Flt3 signaling would lead to inhibition of both dendritic and natural interferon-producing cell development. Using a small molecule tyrosine kinase inhibitor with Flt3 affinity (generous gift from Pfizer/Sugen, CA, USA), we completely blocked dendritic and natural interferon-producing cell development in Flt3-ligand supplemented mouse bone marrow cell cultures, while dendritic cell development in GM-CSF supplemented cultures was not affected. *In vivo* application of this tyrosine kinase inhibitor leads to a substantial reduction of both natural interferon-producing and dendritic cells, comparable to the reduction of these cell types in Flt3-ligand$^{-/-}$ mice. Conversely, Flt3-ligand plasma levels increased massively in inhibitor treated animals, likely via a regulatory feedback-loop, without being able to compensate for pharmacologic Flt3 inhibition. No obvious toxicity is observed. Given the importance of dendritic cells and interferon-producing cells as regulators of immune responses, these findings might lead to new therapeutic strategies in the prevention and treatment of autoimmune diseases and complications of organ or blood cell transplantation. We are now testing this hypothesis in murine models in the setting of experimental autoimmune encephalitis and complete mismatched bone marrow transplantation.

Reconstitution and function of a human adaptive immune system in CD34^{+} cord blood cell transplanted mice (human adaptive immune system Rag2$^{-/-}\gamma c^{-/-}$ mice, huAIS-RG mice)

Laurie Chicha, Elisabetta Traggiai, Roxane Tussiwand, and Markus Manz

Because ethical restrictions limit *in vivo* studies of the human hematolymphoid system, substitute human to small animal xenotransplantation models have been employed. Existing models, however, sustained only limited development and maintenance of human lymphoid cells and rarely produce immune responses. We found that intrahepatic injection of CD34^{+} human cord blood cells into conditioned newborn Rag2$^{-/-}\gamma c^{-/-}$ mice leads to de novo development of B, T, dendritic, and natural type I interferon-producing cells; formation of structured primary and secondary lymphoid organs; and production of some functional immune responses. Using this model, we are now studying in more detail a) human T cell differentiation and selection, b) human *in vivo* IPC, DC, and Langerhans cell differentiation, c) maintenance and differentiation of human hematopoietic stem and progenitor cells, and d) infection, immune responses, and therapeutic interventions in human specific virus infections (EBV and HIV) *in vivo*.

For this study Markus Manz received the Artur-Pappenheim-Prize 2004 from the German Society of Haematology and Oncology.

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T Cell Development

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Denise Ferrera, PhD student 2003
Ursula Schenk, PhD 2005

Former Members
Simona Porcellini, PhD 2002-2005

Introduction

The work of the lab originally focused on the mechanisms implicated in signal transduction by the pre-T cell receptor (pre-TCR). We found that the pre-TCR is constitutively routed to glycosphingolipid-enriched microdomains (rafts) in the plasma membrane of immature thymocyte. This property of the pre-TCR allows spontaneous juxtaposition of the CD3 associated signaling modules with the src-like tyrosine kinase lck, which phosphorylates the ITAMs domains present in such modules (Figure 1).

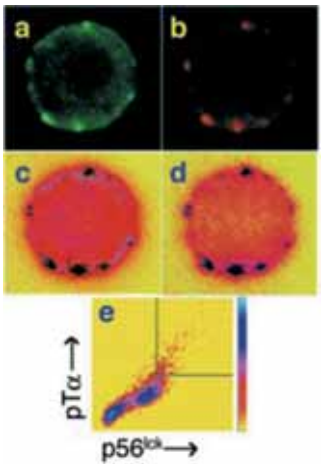

Figure 1. Colocalization of the pre-TCR and the protein tyrosine kinase p56lck. Pre-T cells were stained with p56lck (green) and pTα chain (red) specific antibodies.

Thus, it appears that the pre-TCR is endowed with an exquisite signaling potential that is not dependent on interaction with an exogenous ligand (Saint-Ruf C. et al., 2000). During this study we noticed that the pre-TCR accumulated in lysosomes (Figure 2). As the pre-TCR was hypothesized to signal in a ligand-independent fashion, we reasoned that it could be downregulated constitutively as well. Then, we characterized this constitutive endocytosis and some mechanisms implicated in this process, such as dependence on lck, dynamin and proteasome activities (Panigada M. et al., 2002).

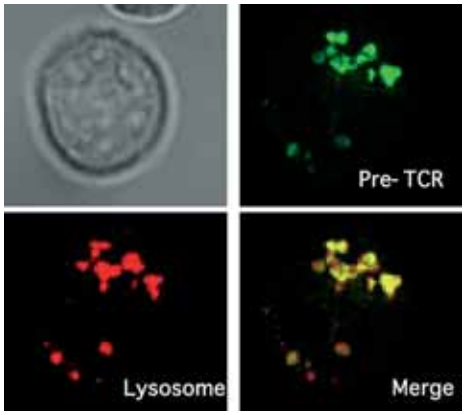

Figure 2. Colocalization of the pre-TCR and a lysosomal marker (Lysotracker).

The signaling characteristics of the pre-TCR are reminiscent of oncogenic receptors, indeed T cell acute lymphoblastic leukemia (T-ALL) was shown to involve pre-TCR expression and signaling.

At the IRB we concentrated our efforts on a model of pre-TCR signaling based on the expression in the plasma membrane of recombinase-deficient (RAG^-) thymocyte of CD3 chains in association with calnexin (CNX), an endoplasmic reticulum (ER) resident chaperone. Once cross-linked this clonotype-independent complex (CIC) was shown to mimic signal transduction by the pre-TCR (Grassi F. et al.,1999). Furthermore, cross-linking of CIC on the surface of RAG^- thymocyte determines cell duplication, phenotypic changes, and gene expression modifications in the thymocyte as well as in the stromal component indis-

tinguishable from the same phenomena induced by expression of the pre-TCR in the physiological context (Guttinger M. et al., 1998; Panigada M. et al., 1999; Porcellini S. et al., 1999; von Boehmer H. et al., 1999) (Figure 3).
Surface CNX is constitutively routed to lysosomes and this downregulation is sensitive to the same drugs as the pre-TCR. We hypothesized that dissection of the molecular requirements of CIC relevant for «pre-TCR like» signaling could be informative on molecular requirements critical for pre-TCR driven development and leukemogenesis. We transfected a SCID thymocyte-derived cell line (SCIET.27) with myc-tagged calnexin (myc-CNX) either unmutated (full lenght, myc-CNXfl) or bearing deletion of the cytoplasmic tail (myc-CNXΔcy) or linked to the plasma membrane through the glycosylphosphatidyl inositol (gpi)-membrane anchor of the Thy-1 molecule (myc-CNXgpi), a strong rafts targeting signal. The various myc-CNXs differently translocated into rafts.
Mutation of the transmembrane or cytoplasmic tail altered the turnover of CNX with stable expression of myc-CNXΔcy and myc-CNXgpi in the plasma membrane implying that constitutive CNX downregulation is not dictated by rafts partition and supporting a crucial role for the cytoplasmic domain in CNX endocytosis. Endogenous CNX expressed in cells transfected with myc-CNXΔcy and myc-CNXgpi was downregulated as in nontransfected cells. In contrast, endogenous and myc-CNXfl were more stably expressed at the cell surface in myc-CNXfl transfectants suggesting that a saturable mechanism active on the cytoplasmic tail is likely responsible for the rapid turnover of surface CNX. The pre-TCR is stabilized in the plasma membrane when expressed in myc-CNXfl transfectants but not in myc-CNXΔcy and myc-CNXgpi transfectants supporting the use of the same endocytic machinery by CIC and the pre-TCR. These observations imply that tailless CNXs are not routed to the endo-lysosomal compartment as is the case for CNXfl and the pre-TCR. These different properties of CNX mutants allow studying the possible dependence on endocytosis for MAP kinase activation by the pre-TCR. Indeed, it was shown for the epidermal growth factor receptor (EGFR) that receptor triggering leads to ERK phosphorylation only if endocytosis is allowed because MEK activation but not p38 activation requires endosome driven scaffolding of signal transduction components. Preliminary results indicate that this is also the case for the pre-TCR and CIC consistent with the crucial role of pTα cytoplasmic tail in supporting pre-T cell development. These results characterise the requirement of endosomal routing for pre-TCR signaling to occur, possibly implying the relevance of such a mechanism in the pathogenesis of pre-TCR dependent leukemias.
Pre-TCR and αβTCR signaling involves cytosolic Ca^{2+} elevations driven by efflux from the ER and influx through Ca^{2+} release activated calcium (CRAC) channels in the plasma membrane. We decided to study the role of calreticulin (CRT), the main Ca^{2+} buffering protein in the ER, in T cell development. Since CRT-deficient mice die during embryonic development we generated foetal liver chimeras in RAG^{-}/common γ chain double knockout (DKO) mice with *crt*$^{-/-}$ as well as *crt*$^{+/+}$ hemopoietic progenitors. These chimeras revealed apparently normal thymic development in the absence of CRT. However, *crt*$^{-/-}$ chimeras displayed a striking phenotype starting at week 7 after reconstitution with alopecia and blepharitis. Moreover, 20% of mice developed a wasting syndrome. Cells displaying markers of activation and constitutively secreting cytokines in both the CD4 and CD8 αβ T cell lineages were increased in peripheral lymphoid organs.

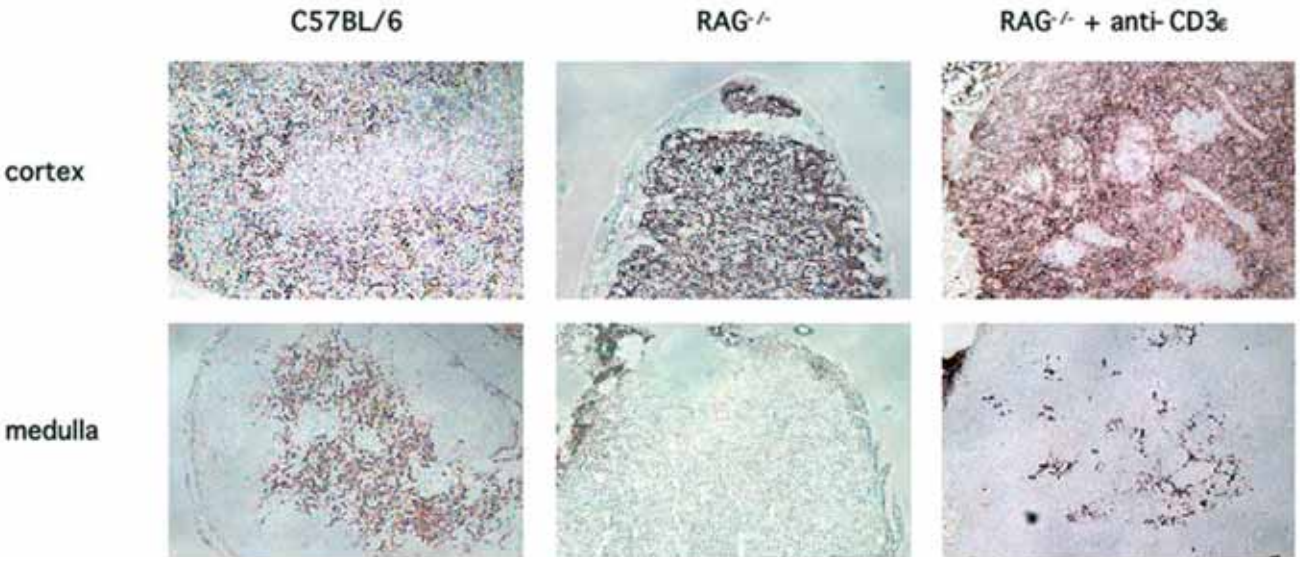

Figure 3. Development of medullary epithelium in the thymus of a RAG- mouse treated with anti-CD3 antibodies.

Analysis of apoptosis of $CD4^+8^+$ thymocytes did not reveal an impairment of *crt*$^{-/-}$ thymocyte to undergo antigen driven clonal deletion, thereby suggesting that tolerance induction through this mechanism was not implicated. The capacity of *crt*$^{-/-}$ $CD4^+CD25^+$ T regulatory cells to inhibit a CD3 driven mitogenic response was not impaired, ruling out an intrinsic defect of this cell subset in determining the immunopathological damage.

Crt$^{-/-}$ cells displayed improved IL-2 secretion upon TCR cross-linking and responsiveness to suboptimal stimulation that does not promote survival of *crt*$^{+/+}$ T cells. Chimeric mice were generated with *crt*$^{-/-}$ progenitors transgenic for the DO11.10 TCR (DO11.10tg) specific for the ovalbumin peptide 323-339 (OVAp). Adoptive transfer of DO11.10tg *crt*$^{-/-}$ cells in normal mice and antigen priming determined exaggerated swelling of the draining lymph node as well as more robust cell proliferation and cytokine secretion than observed with the *crt*$^{+/+}$ counterpart. These results together with the reduced apoptosis and increased IL-2 production by suboptimally stimulated *crt*$^{-/-}$ T cells suggest that the threshold for TCR activation was lowered and persistence of positive signals was enforced in peripheral T cells by CRT deficiency. *Crt*$^{-/-}$ cells displayed Ca^{2+} elevations with higher frequency than *crt*$^{+/+}$ cells and Ca^{2+} elevations were separated by reduced intervals in *crt*$^{-/-}$ vs *crt*$^{+/+}$ cells. Since nuclear factor of activated T cell (NFAT) is dephosphorylated and translocated to the nucleus upon activation of the phosphatase calcineurin by Ca^{2+} signaling we measured dephosphorylated NFAT1 in DO11.10tg *crt*$^{-/-}$ and *crt*$^{+/+}$ T helper clones. These experiments demonstrated an increase in NFAT1 dephosphorylation at steady state as well as a more persistent NFAT1 dephosphorylation in *crt*$^{-/-}$ cells upon CD3ε stimulation, thus suggesting that enhanced IL-2 secretion in *crt*$^{-/-}$ cells could depend on aberrant NFAT regulation. Positive regulators of TCR-induced activation include p38MAPK and ERK. Both kinases were also shown to implement the NFAT transactivation potential, further contributing to the T cell activation genetic program controlled by NFAT. Analysis of phosphorylation at different times after TCR stimulation revealed the protracted activation of both p38MAPK and ERK in *crt*$^{-/-}$ clones, implying a defective control of these T cell activation pathways in the absence of CRT.

This aberrant control of TCR signaling is likely involved in the immunopathology observed in *crt*$^{-/-}$ foetal liver chimeras. Therefore understanding the mechanisms underlying such deregulated T cell response will be informative on the regulatory circuits involved in the control of adaptive immune responses.

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Research Activity

Ongoing projects

Subcellular routing of signals required for pre-T cell development

Denise Ferrera and Fabio Grassi

A peculiar feature of immature thymocyte is to express in the plasma membrane the endoplasmic reticulum (ER) resident chaperone calnexin (CNX) in association with CD3 chains (referred to as CIC, clonotype independent CD3 complex). Calnexin escape from the ER is probably due to less efficient Golgi-ER retrieval machinery in immature thymocyte than in mature T cells. Indeed, we found that PACS-2, a novel sorting protein involved in Golgi-ER retrieval, is developmentally regulated in the T cell with lower expression at the $CD4^-8^-$ double negative (DN) and $CD4^+8^+$ double positive (DP) thy-

mocyte stages when CNX is detected at the cell surface. Plasma membrane CIC partitions to glycolipid enriched microdomains (rafts) as the pre-TCR, albeit to a much lesser extent. Once cross-linked with anti-CD3 antibodies CIC can drive transition of pre-TCR deficient thymocyte to the DP stage mimicking at both cellular and molecular level all events induced by pre-TCR expression. Moreover, surface CIC is constitutively internalized and degraded into lysosomes as is the case for the pre-TCR. Deletion of the cytoplasmic tail of CNX does not affect rafts partition but impairs the turnover of deleted CNX implying that rafts partition per se is not sufficient to induce CNX degradation. Whereas expression of tailless CNX does not affect the turnover of endogenous CNX, over-expression of full length CNX results in poor degradation of both endogenous and exogenous full length CNX. This suggests that the internalization machinery involved in CNX downregulation is saturable. Pre-TCR turn over is affected by over-expression of full length CNX as well, suggesting that CNX and the pre-TCR share the same internalization machinery. Moreover, these findings suggest that the potential of CIC to mimic pre-TCR function could derive from sharing the same subcellular routing of the pre-TCR that is not observed with CNX deletion mutants. Indeed, coculture of recombinase-deficient fetal thymocyte transduced with myc-tagged full lenght CNX with a stromal cell line expressing the Delta 1 ligand for Notch-1 (OP9-DL1) and cross-linking with anti-myc antibodies induced downregulation of CD25, a feature characteristic of pre-TCR expression and signaling.

Role of calreticulin in T cell function

Simona Porcellini, Ursula Schenk and Fabio Grassi

Calreticulin (CRT) is the main Ca^{2+} buffering protein in the endoplasmic reticulum (ER) and its deficiency in mice is embryonically lethal because of altered cardiac development. To study the impact of CRT deficiency on T cell function, we reconstituted recombinase-2-deficient (RAG-2)/common γ chain double knock-out (DKO) mice with fetal liver hemopoietic progenitors (FLP) from *crt*$^{-/-}$ as well as *crt*$^{+/+}$ embryos. RAG/γ chain DKO mice reconstituted with *crt*$^{-/-}$ FLP display alopecia and blepharitis starting at week 7 after reconstitution with 20% of mice progressing to a wasting disease. Cells displaying markers of activation and constitutively secreting cytokines in both the CD4 and CD8 αβ T cell lineages were increased in peripheral lymphoid organs. These cells could derive from inefficient deletion of autoreactive T cells in the thymus; however sensitivity to apoptosis of thymocytes in several assays was unaltered with respect to *crt*$^{+/+}$ cells. A functional defect of the regulatory T cell subset was ruled out by efficient inhibition of anti-CD3 mitogenic response by sorted *crt*$^{-/-}$ $CD4^+25^+$ cells. It was shown that signal strength determines T cell «fitness», i.e. T cells receiving short or weak stimulation of the TCR *in vitro* are not «fit» and die by neglect unless exogenous IL-2 is provided whereas prolonged or strong stimulation promotes fitness by enhancing survival and responsiveness to homeostatic cytokines (IL-7 and IL-15). *Crt*$^{-/-}$ cells display improved IL-2 secretion upon TCR cross-linking and responsiveness to suboptimal stimulation that does not promote survival of *crt*$^{+/+}$ T cells. This aberrant control of TCR signaling is likely involved in the immunopathology observed in *crt*$^{-/-}$ fetal liver chimeras. We investigated Ca^{2+} dynamics in *crt*$^{-/-}$ versus *crt*$^{+/+}$ T cells following TCR αβ stimulation and found that cytosolic Ca^{2+} elevations were significantly perturbed in *crt*$^{-/-}$ cells. *Crt*$^{-/-}$ cells displayed reduced Ca^{2+} release from intracellular stores and increased frequencies of Ca^{2+} elevations after TCR stimulation likely representing inefficient inactivation of Ca^{2+} release activated Ca^{2+} (CRAC) channels. These results suggest that CRT is implicated in the control of T cell activation through the regulation of the calcium response to antigenic stimulation.

In collaboration with Marek Michalak, University of Alberta, Canada, and Michela Matteoli, Università degli Studi di Milano, Italy.

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Swiss National Science Foundation, 3100A0-104237/1, 2004-2007

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Cellular and Molecular Immunology

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Introduction

Lymphocytes are the basic cellular units of adaptive immunity. The goal of our laboratory is to understand how lymphocytes are generated and how do they function. Our developmental model is the mouse thymus where the majority of T cells are produced. We have identified new players in this process and by exploiting genetical, biochemical and cellular approaches we want to gain new insights into T cell maturation and production in the thymus and into T cell function generally. In addition by *in vivo* ablation of specific cellular lineages we want to learn the rules how immune responses and immunological memory are regulated.

Projects

Molecular analysis of TARPP function

Jan Kisielow and Klaus Karjalainen

TARPP is an abundant thymocyte specific protein. It is turned on in precursor cells in the thymus at the moment of commitment to the T cell lineage. It stays on as the thymocytes rearrange their TCR β genes, pass β-selection, expand and rearrange the TCRα genes, to be switched off as a consequence of TCR engagement during positive selection. TARPP is a 100 kDa cytoplasmic protein that contains a R3H domain and has some homology to putative human proteins KIA1002, KIA0029 and to the *Drosophila* protein encore. Encore is implicated in the control of the protein levels of gurken, a protein involved in *Drosophila* oogenesis. The proposed role for the R3H domain is to bind single-stranded nucleic acids. However no experimental data supporting this function has been published. These features suggest a role for TARPP in RNA binding and possibly translational control. Indeed glycerol gradients and gel filtration experiments revealed that TARPP forms a high molecular weight complex with RNA. Expression of truncated forms of TARPP in a thymocyte cell line ScidET mapped the active domain to the N terminal (R3H containing) half of the protein and has shown that TARPP forms multimers. Currently we are trying to isolate and characterize the RNA that TARPP interacts with and verify the role of R3H domain in this interaction. Experiments designed to test whether the other proteins of the "TARPP family" KIA0029 and KIA1002 also interact with RNA are also underway.

Expression of lymphocyte activation gene 3 (LAG 3) on B cells is induced by T cells

Malgorzata Kisielow and Klaus Karjalainen

Lymphocyte activation gene 3 (LAG-3/CD223) is a CD4 homolog known to be selectively expressed in activated T and NK cells. It is thought to have a negative regulatory function in T cells. With the help of new monoclonal antibodies against mouse LAG-3, we show that LAG-3 surface expression is not limited to activated T and NK cells but is also found on activated B cells. Induction of B cell surface expression is T cell dependent and mediated by a soluble factor. The majority of LAG-3 on B cell surface is endogenously produced, even though soluble LAG-3 is present in the culture supernatants and can be passively absorbed. As B cells express LAG-3 in a T cell dependent manner and not when activated by Toll-like-receptor agonists alone, we propose LAG-3 as a new marker of T cell induced B cell activation.

Characterization of a novel T cell specific protein

Zuzana Garajova and Klaus Karjalainen

By screening a subtractive cDNA library from RAG KO thymi we have identified a novel transcript referred to as

Z. Z is highly expressed in the thymus and peripheral T cells but not in other types of cells. Full length cloning revealed two splice variants of Z (Z-1 and Z-3) corresponding to proteins of an estimated molecular weight of 30 and 70 kDa respectively. The Z locus is localized on chromosome 10 in mice and human chromosome 6 contains a highly homologous gene. It spans more than 100kb and is composed of 5 exons. Genebank searches with the Z-3 protein revealed some homology to the basement membrane-induced protein ICB-1. However the similarity was restricted to a short positively charged region, possibly identifying a novel functional domain. Recombinant forms of Z are being produced in order to make monoclonal antibodies for biochemical studies. At the same time targeting constructs for gene deletion are being made.

Characterization of a newly-discovered chemokine

Dominic van Essen and Klaus Karjalainen

We recently identified a previously unknown member of the CXC-chemokine family, which we named X. The origins of X are currently unclear: although its cDNA was originally found in mouse lymphocytes, the gene for X is absent from the mouse genome so far sequenced. Nevertheless, most monoclonal antibodies raised against X stain mouse spleen, but not lymph node, in both western blots and histological sections. The closest relative of X is the mouse gene for platelet basic protein (PBP), which is also predominantly expressed in the spleen; however, antibodies raised against X are all unreactive against mouse PBP. We are currently attempting to use anti-X monoclonal antibodies to purify the protein from mouse spleen, to determine whether it is X itself, or another closely related molecule. To identify a cellular receptor for X, we have joined X to the constant region of human IgG1. The resulting chimaeric protein (X-g1) binds to mouse B cells; however most of this activity results from non-specific sticking to surface proteoglycan molecules. We are now using a mutated form of X-g1, which does not bind proteoglycans to screen expression libraries derived from various mouse tissues. In vivo, X is located within the red pulp and marginal zones in the spleen, and *in vitro* it is a chemoattractant for T cells, but its role is a mystery. Injection of X-g1 into mice, which may interfere with its normal function, results in an acceleration of antibody production during an immune response. We do not yet know the cellular mechanism for this.

Genomic surprises

Piotr Tetlak and Klaus Karjalainen

We have recently identified two partial transcripts which are both expressed abundantly only in thymus and testis. Interestingly 3' ends of those transcripts map, in reverse orientation, 15 kb from each other in a completely sequenced genomic piece. Since exon/intron prediction algorithms did not provide satisfactory information we have started to identify the potential exons by using exon-trap technology and thus far at least 5 exons for both genes have been located. Surprisingly there is at least two exons' overlap between these genes. Preliminary analysis has showed that human genome contains highly homologous and also similarly complex region supporting the notion that the observed complexity is not of accidental nature.

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Chemokine Tissue Expression, Function and Activity Modulation

Molecular Interactions of Chemokines

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Introduction

Motility is a hallmark of leukocytes. This property is of crucial importance for all aspects of immunity and forms the basis for hematopoiesis and immune defence. Breakdown in the control of leukocyte mobilization contributes to the pathogenesis of chronic inflammation as well as of tumour development. It is now generally accepted that leukocyte trafficking in homeostasis as well as in pathology is largely determined by the more than forty chemokines that are produced constitutively or upon specific induction in virtually all tissues of the human body, in combination with the expression of almost twenty target receptors on all leukocyte subsets and on many tissue cells (Figure 1).

Although much remains to be discovered, the receptor specificities of most chemokines, the expression patterns of chemokine receptors, and the resulting immunological activities are often known in intricate detail from numerous *in vitro* and *in vivo* studies. While we comprehend well the effects of chemokines one by one, much less is known about the potential consequences of multiple and concomitant chemokine expression on leukocyte migration and function, even though numerous *in situ* experiments clearly document the simultaneous expression of several or many chemokines at diverse target sites of leukocyte trafficking and homing. Evidence from other and our own groups has recently revealed the existence of additional mechanisms that apply under conditions of multiple and concomitant chemokine expression.

Natural Chemokine Antagonism

The term «natural antagonist» designates endogenous chemokines that feature inhibitory activities distinct of and in addition to their agonistic properties (Ogilvie et al., 2003; Ogilvie et al., 2001; Petkovic et al., 2004a; Petkovic et al., 2004b). Such natural antagonists also include chemokines that have acquired their inhibitory properties by protease modification, as well as viral chemokine homologues with inhibitory potential.

Protease-modified chemokines – While chemokines are very resistant to proteolytic degradation and inactivation in general, specific processing can occur in the N termi-

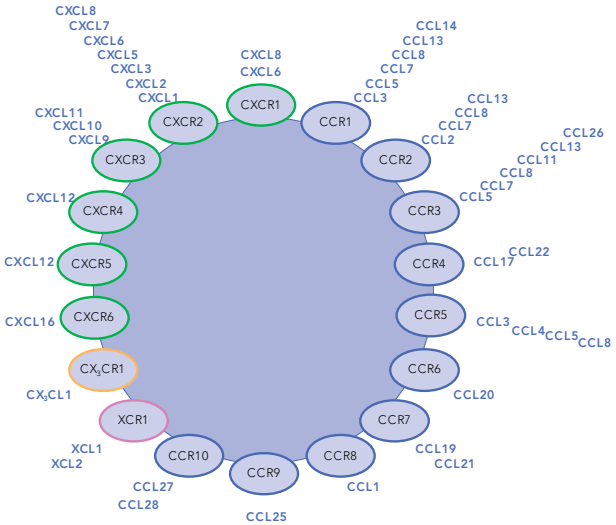

Figure 1. The human chemokines and chemokine receptors.

nal and C terminal domains. Various enzymes, namely dipeptidyl peptidase IV (DPP IV/CD26) and matrix metalloproteinases (MMPs), can process chemokines, thus generating inactive chemokines, chemokine antagonists, and chemokines with altered receptor selectivity or increased activity.

Native chemokines – The two-site model of chemokine receptor binding and activation implies that a native chemokine featuring a matching binding domain and a «mismatched» activation domain might act as an antagonist for a particular receptor just as well. Altogether, nine native chemokines are currently known to have inhibitory activities apart from their previously known agonism. Most show narrow antagonist specificity, inhibiting only one receptor. Notable exceptions are CXCL11 and CCL26, which are specific agonists for CXCR3 and CCR3, respectively, but inhibit two (CCR3 and CCR5) and three (CCR1, CCR2, and CCR5) receptors, respectively. The current data suggest the notion that endogenous chemokines inhibit their target receptor by competitive antagonism, much as it is known for many other G protein coupled receptors.

In vivo relevance - N-terminally truncated, synthetic as well as protease-modified chemokine antagonists have previously demonstrated their antagonistic potential *in vivo* in several rodent models. More recently, the native form of CXCL9, a somewhat modest CCR3 inhibitor *in vitro*, was found to be an efficient *in vivo* antagonist as well.

Natural Chemokine Synergism

An abundant number of publications describe various forms of synergism between different pro-inflammatory substances, cytokines, chemoattractants and chemokines, involving many growth hormone, cytokine, Toll-like and G protein coupled receptors (Panzer et al., 2004). The synergistic combinations of chemokines and chemoattractants seem to act via two different mechanisms. On one hand, chemokine synergism may be due to intracellular priming events. On the other hand, chemokines seem to be capable of forming heteromeric complexes that are more active than the single chemokines or their homomeric complexes themselves.

Chemokine synergism by intracellular priming events – *R*egakine, a bovine chemokine with no known human orthologue to date, can specifically increase the activity of certain chemokines and chemoattractants such as CXCL6, CXCL7, CXCL8, fMLP, and C5a. An analogous synergism has been shown for CXCL8 in the presence of CCL2, CCL7, CCL8, and CXCL12. Chemotaxis, MMP-9 secretion and cellular adhesion are all enhanced in hematopoietic stem/progenitor cells with a combination of C3a and CXCL12. Another, reciprocal synergism modulates the responses of CXCR3 and CXCR4 to their agonists: CXCL12 primes the responsiveness of $CXCR3^+$, natural IFN-producing cells to CXCL9, CXCL10, and CXCL11, while the reactivity of $CXCR4^+$ to CXCL12 is similarly increased in the presence of CXCL9, CXCL10, and CXCL11. The nature of the priming mechanism causing the synergistic events remains to be determined for all of these systems, however.

Chemokine synergism by heteromeric chemokine interactions – By nuclear magnetic resonance and plasmon resonance-based Biacore analysis, two recent reports clearly demonstrate that CXCL4 and CXCL8 form heterodimers that were more active in hematopoiesis and chemotaxis assays than the respective chemokines on their own. CXCL4 and CXCL8 interact via their , sheets, akin to how their homomeric complexes form. CXCL4 interacts also with CCL5 in a heteromeric and synergistic way, increasing monocyte adherence on activated endothelial cells. Synergism induced by heteromeric chemokine complexes may well be a widespread but nevertheless specific phenomenon, as documented by the large number (20 out of 25 tested) of chemokines that synergistically increase the action of CCL19 and CCL21 on CCR7. Apart from chemotaxis, CCR7 internalization and ERK phosphorylation are synergistically increased as well. Western blot and binding experiments again show the formation of heteromeric complexes suggesting these complexes as the cause of the observed synergism (Paoletti et al., 2005). Similar synergy mechanisms enhance CCR4 responses towards CCL17 and CCL22. Interestingly, chimeric mutants between two chemokines with (CCL7) and without (CCL4) synergistic activity imply that residues in the first, strand mediate heteromeric association and synergism, much in analogy to the interaction between CXCL4 and CCL5 (Sebastiani et al., 2005).

Synergism by heteromeric chemokine interactions may be a widespread phenomenon, positively regulating diverse chemokine activities such as chemotaxis, cellular adherence, receptor internalization, and protein kinase phosphorylation. Interestingly, the available structure and structure-function data, albeit scarce to date, collectively implicate residues in the first, strand as mediators of heteromeric association and synergism. It is thus tempting to speculate that heteromeric chemokine complexes may mimic those homomeric dimers that form via association of their, sheets, featuring an interface composed of the first, strands. However, the molecular reasons as to why a heteromeric complex should be more active than a homomeric one remain, at present, completely obscure. Certainly, speculating that heteromeric chemokine association might promote receptor heterodimerization, which was reported to increase receptor activities, would constitute an attractive hypothesis. Surface representations with electrostatic potentials of

chemokines show similarities among selective agonists and known natural antagonists, thus indicating this analysis as an additional instrument for disclosing the potential of different chemokines as natural antagonist or synergy inducing molecules (Figure 2).

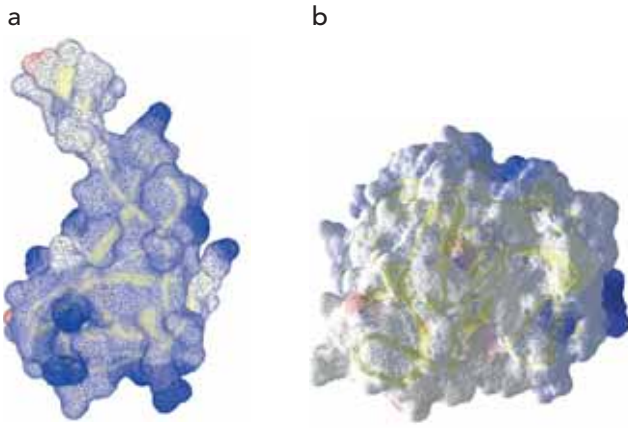

Figure 2. a) Surface representation with electrostatic potential of MCP 1 with the backbone ribbon in yellow. The flexible N terminus is at the top, and the C terminal α-helix in the back. b) Surface representation with electrostatic potential of CCR2 with the backbone ribbon in yellow, as seen from the outside of the cell. The seven α-helices are arranged counter-clockwise with the first helix at the lower right.

Chemokines in pathology

A vast literature documents the expression of various chemokines in inflamed tissue, as well as distinct chemokine receptor expression by infiltrating cells (Gerber et al., 1997; Loetscher et al., 1998). Nevertheless, the real significance of multiple chemokine expression in inflammation is a controversial issue. Studies on chemokine expression in human tissue samples from different diseases have revealed that resident and infiltrating cells can produce a variety of inflammatory chemokines (Figure 3). Lymphoid microarchitectural features forming in a chronically inflamed tissue outside the constitutive environment of secondary lymphoid organs might represent the extreme attempt of the immune system to eradicate a local persistent antigenic stimulation. However, the aberrant architecture and the ectopic localization, together with the large amounts of antigen locally available, rather than eradicate the process, would result in breaking of self-tolerance, perpetuation of the immune/inflammatory activity and amplification of effector mechanisms (Manzo et al., 2005; Mazzucchelli et al., 1999; Smith et al., 2003).

Research in the chemokine field has dramatically changed our understanding of leukocyte traffic in immune defence and disease, offering attractive perspectives for new therapeutic approaches in the treatment of

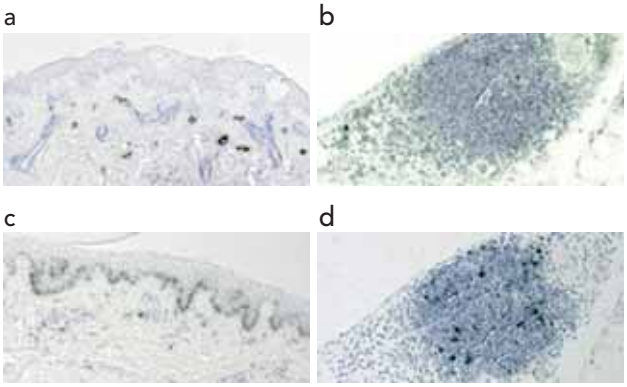

Figure 3. Analysis of chemokine mRNA by in situ hybridization. SLC/CCL21 (a) and MCP-1/CCL2 (c) in a skin sample of human contact dermatitis (magnification 10x); SLC/CCL21 (b) and BCA-1/CXCL13 (d) in a synovial membrane sample of human Rheumatoid arthritis (magnification 20x).

chronic inflammation and infectious diseases. The therapeutic potential of modulating chemokine activities was recognized from the beginning, however. Early studies with receptor antagonists obtained by modifying the structure of natural chemokines proved the full validity of this concept, and low-molecular weight chemical compounds were recognised as prototypes for anti-chemokine drugs.

There are clear indications for a role of chemokines in tumour biology, but the study of this area is still in its beginning. High-levels of multiple chemokines are expressed in tumour cells, tumour tissues and transformed cell lines (Figure 4).

It has been suggested, and in some circumstances shown, that chemokines can act as growth factors and may have angiogenic or angiostatic properties. Tumours could also express chemokines in order to weaken immune defence. More recently it has also been shown that chemokines may mediate tumour cell migration and metastasis, suggesting that chemokine receptors expressed on tumour cells are novel targets in anti-tumour treatment.

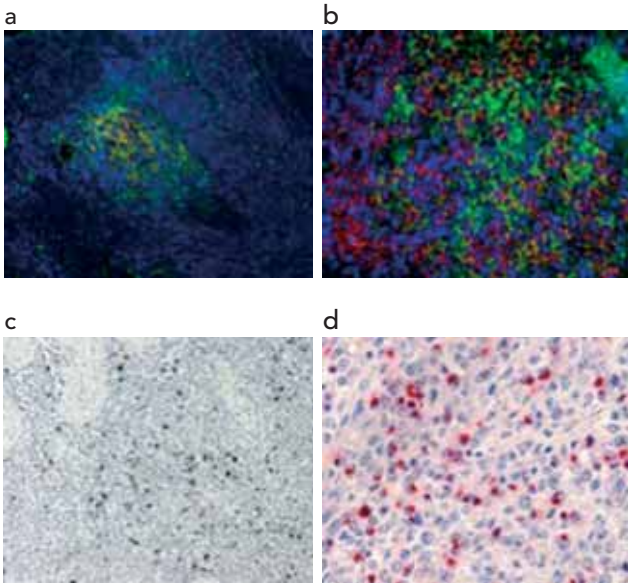

Figure 4. BCA-1/CXCL13 expression in DLCL lymphomas of extranodal sites. Immunofluorescence analysis of BCA-1/CXCL13 (green) and CD21 (red) in thyroid (a) (magnification 10x); BCA-1/CXCL13 (green) and CD20 (red) in the skin (b) (magnification 20x); In situ hybridization analysis of BCA-1/CXCL13 mRNA expression in the testis (c) (magnification 10x); Immunohistochemical analysis of BCA-1/CXCL13 expression in the testis (d) (magnification 80x).

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Ongoing projects

Impact of multiple chemokine expression in human disease

Katrin Kuscher and Mariagrazia Uguccioni

It is undoubted that chemokines are among the key components controlling leukocyte traffic and function. However, very little is known about the consequences of multiple chemokine expression in physiology and pathology. There is a complex mix of several chemokines expressed together in inflammation. In this milieu, the activities of individual chemokines can be modified leading to a dramatic change of leukocyte migration and function. Hence, it is critical to understand the mechanisms of cross regulation between chemokines in order to understand more fully the role of these important regulators. It will then be possible to design highly effective and specific new therapies for inflammatory diseases. We have identified chemokines that – beyond their agonistic activityies – can act as natural antagonists for different chemokine receptors, have a repulsive effect on leukocyte subtypes, or enhance selective chemokine induced-responses. We extend our ongoing research to identify chemokines which provide an additional level of regulation in leukocyte traffic and activation. Specifically: i) We characterize in detail the activity of chemokines that can provide an additional level of control of leukocyte responses. ii) We extend the characterization of the expression of chemokines in selected human tissue samples.

Vaccines against simian immunodeficiency viruses based on deletion of the *nef* gene (SIVΔ*nef*)

Maria Gabriela Danelon, Silvia Sebastiani, and Mariagrazia Uguccioni

Simian immunodeficiency virus (SIV) infection of rhesus macaques provides a model for HIV infection in humans and represents the ideal system to evaluate whether vaccination against SIV blocks infection at the site of viral entry, including mucosal surfaces. Most efforts have been directed to the long-term effects of the vaccine. It is now clear that early events during the entry of a vaccine or challenge virus are readily amenable to analysis in tonsils, which are a model for transmission through mucosa-associated lymphoid tissue (MALT), e.g., in anal transmission by means of rectal MALT. We participate in a project involving several laboratories in Europe and North America to analyze the portal of entry and to track the spread of SIV infection via the lymphatic system to the lymphoid organs. We have found that vaccination of rhesus macaques with the attenuated virus SIVΔ*nef* induces the formation of long lasting thymic lymphoid follicular hyperplasia. These follicles are detectable even after more than a year following infection with SIVΔ*nef*. We have shown that follicle-like structures at ectopic sites (Rheumatoid arthritis, *Helicobacter Pylori*-induced gastritis) are associated with the expression of BCA-1/CXCL13 and SLC/CCL21. We are currently investigating the expression of these two chemokines, and characterizing the producing cells, in the thymus of vaccinated as well as infected animals.

Vaccines against human and simian immunodeficiency viruses: characterization of chemokine and chemokine receptor expression in lymphoid organs

Maria Gabriela Danelon and Mariagrazia Uguccioni

Knowledge about changes in number, activation and distribution of lymphoid organ leukocytes is the basis for understanding immunological changes that occur during infection, and for developing efficient vaccines. The distribution of lymphocytes according to their chemokine receptor expression upon vaccination and/or after infection will be analysed in secondary lymphoid organs of rhesus macaques by immunohistochemistry and *in situ* hybridisation. It has been reported that NK cells circulate in blood but are largely excluded from mouse lymph nodes under steady-state conditions. However, some circulating NK cells express the lymph node homing receptors CD62L and CCR7. A recent report has shown that NK cells are rapidly recruited to lymph nodes that are undergoing an immune response. Moreover, NK cell recruitment is mediated also by CXCR3 and at these sites NK cells can provide an initial source of IFN-γ, and CCR5 ligands. We analyze the presence and distribution of CXCR3$^+$ leukocytes, IFN-γ^+ cells as well as the expression pattern of inflammatory chemokine in the lymphoid organs of rhesus macaques vaccinated and/or challenged with wild type SIV. Chemokine and chemokine receptor expression patterns will be compared with the pattern observed in human normal and reactive lymph nodes, as well as in lymph nodes from HIV positive patients.

Analysis of the B cell memory repertoire of patients with autoimmune disease

Barbara Paviglianiti, Antonio Lanzavecchia, and Mariagrazia Uguccioni

Autoimmune disease is the result of complex interactions between T lymphocytes, B lymphocytes and professional antigen-presenting cells, such as macrophages and dendritic cells. These cellular interactions result in autoaggressive responses that, in different tissues and organs, target a number of different cell types. Although the aetiology of most autoimmune diseases is unknown, recent years have witnessed important advances in understanding the mechanisms leading to autoimmune inflammation and tissue damage. Cytokines and chemokines orchestrate the recruitment, survival, expansion, effector function, and contraction of autoreactive lymphocytes in autoimmunity, contributing to initiation and progression of the inflammatory process. Thus, proinflammatory cytokines and chemokines, as well as their receptors, constitute an important target for therapeutic intervention. The present project will concentrate on the detection of anti-cytokine, -chemokine, and lymphocyte surface molecules in the sera of patients with autoimmune disease. Once the patients with the antibodies of interest will be identified, blood B cells will be isolated and cloned. The antibodies will then be produced in vitro and characterized for their ability to inhibit leukocyte functions upon cytokine or chemokine triggering. The last part of this study aims at identifying the potential of anti-cytokine or –chemokine antibodies to counteract the inflammatory reaction and to open new perspectives for novel immunotherapy in autoimmune diseases.

Chemokine expression in primary central nervous system lymphoma

Maria Gabriela Danelon and Mariagrazia Uguccioni.

Primary central nervous system lymphoma (PCNSL) is a rare form of extranodal non-Hodgkin's lymphoma that accounts for 4% all primary brain tumours. Its incidence is increasing steadily since the 1970s, mainly in immunosuppressed patients, but also in immunocompetent individuals. In the latter population, the reason for the increased

incidence is less clear. In both contexts the number of patients with PCNSL will likely increase over the next decade. PCNSL is a very aggressive tumour with a poor prognosis. To understand the specific issues related to the treatment of PCNSL, we must first understand the underlying unique biology of this tumour. Current biologic knowledge of PCNSL is still largely unknown and several fundamental questions remain unanswered. Under the sponsorship of Oncosuisse the project intends to improve our knowledge of PCNSL pathogenesis and management. This will be done by a strict collaboration among clinicians, pathologists, and scientists. Besides the properties of lymphoma cells, recent data suggest that the characteristics of the microenvironment might play a fundamental role in PCNSL pathogenesis. We will be involved in this study of the PCNSL pathogenesis, analysing chemokine and chemokine receptor expression in tissue samples. An open question is the mode used by neoplastic cells to get to cerebral parenchyma. We have demonstrated that PCNSL cells, as well as vascular endothelium are a primary source of one chemokine, the B-cell-attracting chemokine 1 (BCA-1/CXCL13). At present only scattered information is available on chemokine expression and activity in B cell lymphomas at both nodal and extra-nodal sites. Nevertheless, there are clear indications for a role of chemokines in tumour biology. Finally, the recent demonstration of the presence of low amounts of B lymphocytes in normal brain opens new insights in the pathogenesis of PCNSL, and support the *in situ* study on the expression of homeostatic as well as of inflammatory chemokines.

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Cellular Immunology

Laboratory

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Introduction

Specific immune responses require the timely interaction of various cell types within specific microenvironments (Sallusto et al., 2000). In the primary response the rare antigen specific naive T cells need to maximize the possibility of encounter with antigen. They do so by continuously recirculating through secondary lymphoid organs where they are stimulated by antigen-presenting mature dendritic cells (DCs). Soluble antigens can reach the lymph node directly but in most cases they are carried by migrating DCs that capture antigen in peripheral tissues and subsequently move through the lymphatics to the draining lymph node. Migrating DCs therefore represents packets of information for T cell priming. One goal of our laboratory is to understand how their numbers and activation state may impact on specific events in the lymph node and in general on T cell priming.

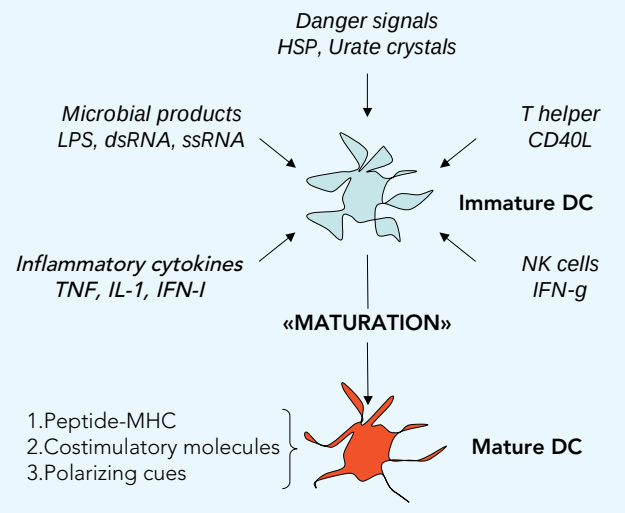
Recognition of specific antigen on mature DCs leads to T cell proliferation and differentiation to effector cells that acquire the capacity to migrate into peripheral inflamed tissue where they eliminate the invading pathogens directly or through activation of effector leukocytes. A long standing interest of our laboratory has been to dissect the signals by which DCs determine differentiation of proliferating T cells towards the T_H1 or T_H2 lineage and how migratory capacity and effector function are coordinately regulated in developing T cells (Lanzavecchia and Sallusto, 2001).

Some of the expanded T cells following antigenic stimulation survive as memory cells. We have originally characterized two subsets of memory T cells based on their migratory capacity and effector function (Sallusto et al., 2004). Central memory T cells (T_{CM}) express homing receptors for lymph nodes and have no or low level effector function. In contrast effector memory T cells (T_{EM}) lack lymph node receptors and have immediate effector function. We have been interested in understanding the molecular basis underlying the functional properties and the differentiation potential of T_{CM} and T_{EM} and in further dissecting their heterogeneity. We are also interested to define the composition of memory subsets in different pathological and physiological conditions to gain insights into the role these subsets play in the immune responses.

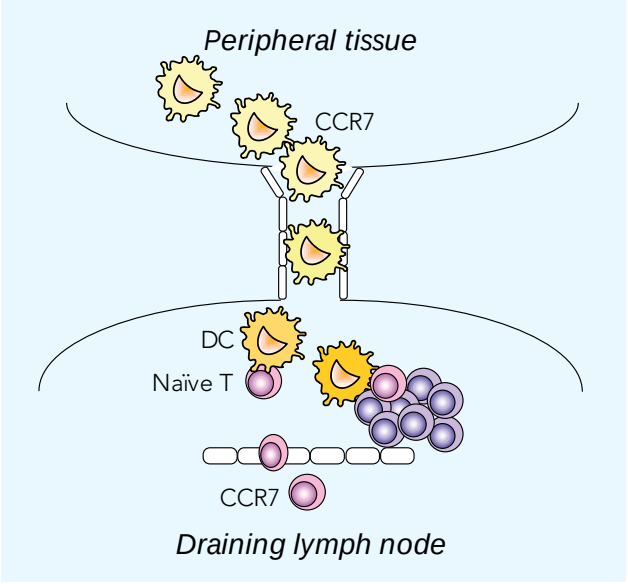
T cell priming by DCs: the role of maturation stimuli and kinetics

To prime immune responses, DCs need to mature in order to acquire T cell stimulatory capacity and the capacity to release cytokines, such as IL-12, that contribute to determine the class of T cell response generated. We originally developed a method for generation of immature DCs from monocytes using GM-SCF and IL-4 (Sallusto and Lanzavecchia, 1994). This method has been instrumental to identify the nature of the stimuli that induce and modulate DC maturation. In response to maturation stimuli DCs downregulate macropynocytosis and endocytic activity and upregulate MHC and costimulatory molecules (Sallusto et al., 1995). Maturing DCs release different types of chemokines and cytokines in response to specific stimuli and with distinct kinetics (Sallusto et al., 1999c; Langenkamp et al., 2000).

In particular we have shown that DCs produce IL-12 only transiently and become refractory to further stimulation. Thus, soon after stimulation «active» DCs prime strong T_H1 responses, whereas at later time points the same cells, which we defined «exhausted», preferentially prime T_H2 and nonpolarized central memory-like T cells. We have proposed that during an immune response, T cell priming conditions may change in the



Integration of multiple signals by dendritic cells



CCR7 orchestrates the encounters of dendritic cells and naive T cells in lymph nodes

draining lymph nodes as a function of DC migration and activation state (Lanzavecchia and Sallusto, 2000). At early time points the high numbers of active DCs may prime effector cells whereas at later time points low numbers of exhausted DCs and competition prime central memory or T_H2 cells.

In vivo dynamics of T cell priming by DCs

Migration of antigen-carrying DCs from tissue to draining lymph node is a key event in determining T cell priming. We have investigated in a mouse system the parameters that affect the migration of subcutaneously injected mature DCs to the draining lymph node (Martín-Fontecha et al., 2003). We found that the efficiency of DC migration varies with the number of injected DCs and that $CCR7^{+/+}$ DCs migrating to the draining lymph node, but not $CCR7^{-/-}$ DCs that fail to do so, efficiently induce a rapid increase in lymph node cellularity, which is observed before the onset of T cell proliferation. The efficiency of DC migration can be boosted up to 10-fold by conditioning the injection site with TNF that acts by increasing the expression of CCR7 ligand CCL21 on lymphatic endothelial cells. Furthermore, the magnitude and quality of $CD4^+$ T cell response is proportional to the number of antigen-carrying DCs that reach the lymph node and can be boosted up to 40-fold by tissue conditioning.

In the same experimental system we have also shown that mature DCs reaching the draining lymph node promote recruitment of NK cells from peripheral blood (Martín-Fontecha et al., 2004). Migration of NK cells into lymph node is CCR7-independent, CXCR3 dependent, can be induced by some, but not all adjuvants and correlates with the induction of T_H1 responses. By performing NK cell depletion and reconstitution experiments we have shown that NK cells provide an early source of IFN-γ that is necessary for T_H1 polarization. These results reveal that DC migration controls both quantitative and qualitative aspects of the immune response. These concepts provide rational basis for the improved use of DCs in therapeutical settings and some mechanistic insights into the role of adjuvants.

Regulation of migratory capacity and effector function in $CD4^+$ T cells

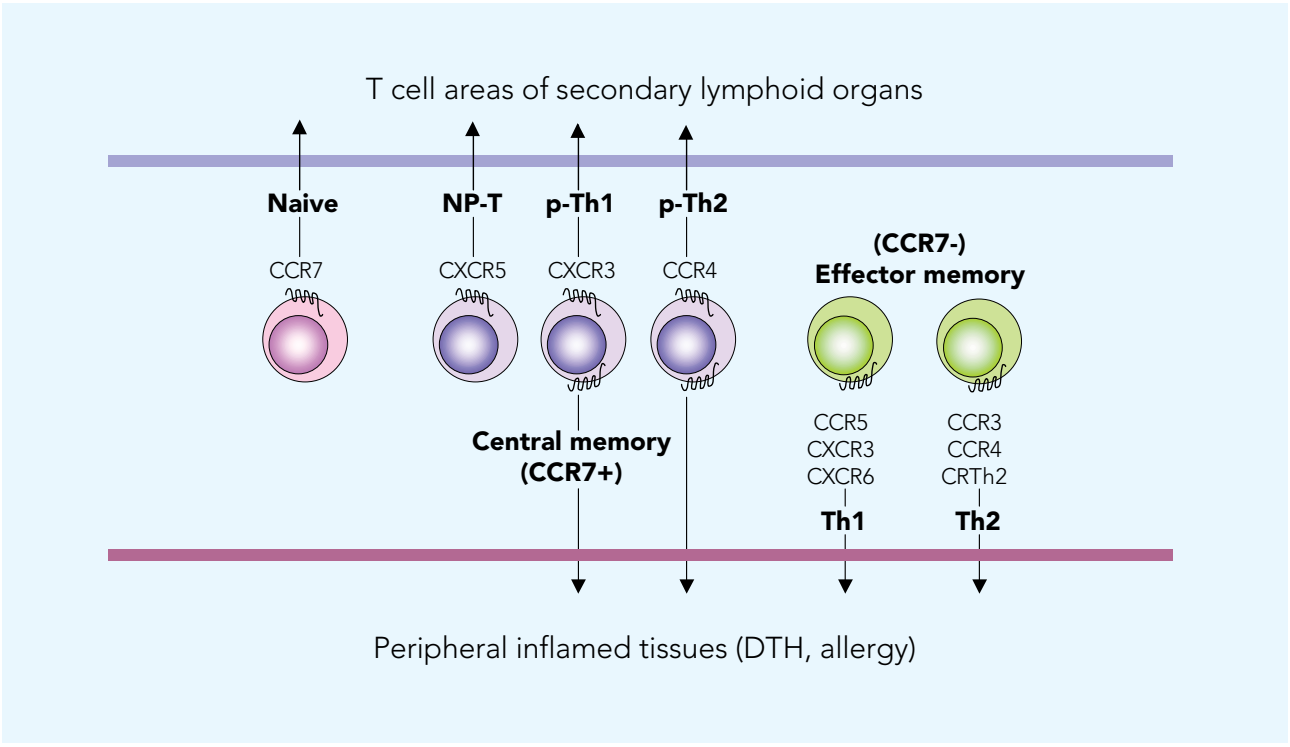
Upon appropriate stimulation, naive T lymphocytes differentiate to T_H1 or T_H2 that mediate different type of immune response to different pathogens. While in mouse cells T_H1 and T_H2 cells are thought to represent terminally differentiated cells that are irreversibly committed to either the T_H1 or T_H2 lineage, we found that human T_H1 and T_H2 cells possess both cytokine memory and flexibility. Indeed, when stimulated under neutral conditions human T_H1 or T_H2 cells retain their polarization, demonstrating that the pattern of cytokine gene expression that is imprinted at priming can be stably maintained (Messi et al., 2003). However, when stimulated under opposite polarizing conditions most polarized T cells can acquire the capacity to express the alternative cytokine. Thus, T_H1 maintain the IFN-γ producing capacity but also acquire the capacity to produce IL-4. Similarly, most T_H2 maintain IL-4 production but acquire IFN-γ producing capacity. An exception is represented by $CRTh2^+$ T cells, which are irreversibly committed to the T_H2-lineage and are unable to acquire IFN-γ-producing capacity when stimulated with IL-12. Considering that in

humans most memory T cells maintain cytokine flexibility, it is possible that expression of the opposite cytokine can be enforced by delivering recall antigens together with the appropriate polarizing signals.

The effector function of T_H1 and T_H2 cells depend on their capacity to produce effector cytokines and to migrate to inflamed tissue together with effector cells such as neutrophils and macrophages in the case of T_H1, or eosinophils and basophils in the case of T_H2. Work from our and other laboratories demonstrated that migratory capacity and effector function are coordinately regulated in effector T cells (Sallusto et al., 1998a). We originally reported that T_H1 and T_H2 cells express different sets of chemokine receptors which are shared with the leukocytes with which they colocalize in the inflamed tissue (Sallusto et al., 1997; Sallusto et al., 1998b; Sallusto et al., 1998c). Thus, T_H1 express primarily CXCR3 and CCR5 while T_H2 cells express CCR3 and CCR4. This pattern which is characteristic of resting cells is rapidly and transiently switched upon TCR stimulation resulting in downregulation of receptors for inflammatory chemokines and upregulation of the receptor for lymphoid chemokines CCR7 and CXCR5 (Sallusto et al., 1999a). The kinetics of chemokine receptor expression has been studied in naïve T cells stimulated by different DC subsets (myeloid or plasmacytoid) and under T_H1 or T_H2 conditions. This analysis revealed that some chemokine receptors are rapidly upregulated before division in all conditions of stimulation whereas other are selectively induced at later time points depending on the T_H1 or T_H2 stimulatory condition or DC type (Langenkamp et al., 2003).

Central memory and effector memory T cells

Memory is the hallmark of the acquired immune system. It results from the clonal expansion and differentiation of antigen-specific lymphocytes that ultimately persist for a lifetime. Memory lymphocytes confer immediate protection in peripheral tissues and mount recall responses to antigens in secondary lymphoid organs. In the B cell system these functions are carried out by distinct cell types. «Protective memory» is mediated by plasma cells that secrete antibodies, while «reactive memory» is mediated by memory B cells that proliferate and differentiate to plasma cells in response to secondary antigenic stimulation. A similar division of labour has recently emerged in the T cell system (Sallusto et al., 1999b). According to the model proposed (Lanzavecchia and Sallusto, 2000), protective memory is mediated by effector memory T cells (T_{EM}) that migrate to inflamed peripheral tissues and display immediate effector function, while reactive memory is mediated by central memory T cells (T_{CM}) that home to T cell areas of secondary lymphoid organs, have little or no effector function, but readily proliferate and differentiate to effector cells in response to antigenic stimulation (Sallusto et al., 1999b).



Human T cell subsets identified by chemokine receptor expression

A sizable proportion of circulating T_{CM} expresses CXCR5, the receptor for CXCL13, a chemokine produced in B cell follicle (Breitfeld et al., 2000). These $CXCR5^+$ T cells, which have been defined as follicular helper T cells (T_{FH}), are non-polarized cells and upon activation produce IL-2 and some IL-10. When analyzed at the clonal level T_{CM} stimulated *in vitro* appear to be heterogeneous as far as their capacity to differentiate (Messi et al., 2003). Some cells can be propagated in a non-polarized state by stimulation under neutral conditions, and can be induced to differentiate to T_H1 and T_H2 upon stimulation in the presence of IL-12 or IL-4, respectively. Others, however, spontaneously differentiate to IFN-γ or IL-4 producing cells, even if stimulated in the absence of polarizing cytokines. These cells represent pre-T_H1 and pre-T_H2 and can be identified according to the expression of CXCR3 and CCR4, respectively (Rivino et al., 2004). In primed individuals antigen-specific CD4 and CD8 T cells can be detected in both T_{CM} and T_{EM} (Sallusto et al., 1999a; Dunbar et al., 2000; Rivino et al., 2004). In the case of tetanus toxoid specific CD4 T cells can be detected in the two memory subsets up to ten years after antigenic stimulation and their frequencies increase in both subsets following booster immunization. Conversely, recall responses to cytomegalovirus and vaccinia virus are largely restricted to $CXCR3^+$ T_{CM} and T_{EM} cells.

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Ongoing projects

Characterization of a cyanobacterial glycolipid that protects against septic shock and suppresses the LPS-induced inflammatory response in dendritic cells

Annalisa Macagno and Federica Sallusto

Microbial infections induce chemokine and cytokine cascades that coordinate innate immune defense. Although inflammatory responses are essential for eradicating invading pathogens, excessive and prolonged responses are detrimental to the host and, in some cases, even fatal, owing to severe tissue damage and circulatory failure. To prevent such an undesirable outcome, proper gating of activation of innate immunity as well as induction of negative feedback regulation, are crucial. In pursuit of the identification of natural compounds with immunomodulatory properties, we extracted from Cyanobacteria a glycolipid that we named VB3320.1 (VB). In *in vitro* assays on human monocytes and DCs, VB is a potent antagonist of proinflammatory stimuli acting through Toll Like Receptor 4. Specifically, it inhibits activation of the MAP kinases JNK and p38 and of NF-κB, with subsequent suppression of cytokine and chemokine gene transcription. Importantly, VB injected together with LPS or bacteria is able to protect mice against lethal endotoxic shock. These results open promising perspectives for the use of VB as a therapeutic agent able to control innate immune responses. Ongoing experiments are defining the chemical structure of VB and tempting to unravel the mechanism at the basis of its inhibitory properties.

In collaboration with Carlo Rossetti and Monica Molteni, University of Insubria, Varese, Italy and Siegfried Morath and Thomas Hartung, University of Konstanz, Konstanz, Germany.

Cell migration in antigen-stimulated lymph nodes and the control of adaptive immune responses

Alfonso Martín-Fontecha, Miroslav Hons, Greta Guarda, Antonio Lanzavecchia, and Federica Sallusto

We have recently found that the constitutive cell traffic in lymph nodes is perturbed when the lymph node is stimulated by migrating mature DCs. In particular NK cells and $CCR7^-$ T cells are efficiently recruited to stimulated lymph node whereas they are excluded from the controlateral lymph node. Cytokines produced by recruited NK cells influence T cell priming and the development of the immune response. We are using a variety of approaches to dissect the mechanisms responsible for the inducible pathway of cell migration in antigen-stimulated lymph nodes and explore the consequence of this perturbed migration on the extent and quality of effector and memory T cells generated. We have hypothesized that in secondary responses T_H1 or T_H2 cytokines produced by activated effector memory T cells may participate in the differentiation of naïve and central memory T cells in antigen-stimulated lymph node. We are comparing different adjuvants as well as DCs activated by a variety of TLR ligands for their capacity to recruit NK cells and other leukocytes in draining lymph nodes. In parallel we are measuring the T cell responses induced by the different adjuvants and follow the development of effector and memory T cells *in vivo*. For these experiments we are using a variety of surface markers that distinguish subsets of activated and memory T cells, including chemokine receptors (CCR7, CXCR4, CCR5), adhesion molecules (L-selectin), and cytokine receptors (IL-7R and IL-15R). To analyze cell rolling and adhesion to high endothelial venules (HEV) in stimulated lymph nodes we will use an intravital microscope platform focused on lymph node microcirculation and low order venules. This work is done in collaboration with Christine M'Rini and Jean-Philippe Girard, IPBS-CNRS UMR 5089, Toulouse. France.

T cell priming by active and exhausted DCs in vivo

Miroslav Hons, Alfonso Martín-Fontecha, and Federica Sallusto

In vivo the strength of T cell stimulation is determined by the nature of DC-T cell interaction. It has been demonstrated that immature DCs have low T cell stimulatory capacity, engage T cells in short contacts and are tolerogenic whereas mature DCs are highly stimulatory and consequently efficiently prime naive T cells. However, while recently activated mature DCs secrete polarizing cytokines such as IL-12 and engage T cells in stable contacts, mature DCs at later time points fail to provide polarizing stimuli and engage T cells in transient interactions. We hypothesized that while immature DCs provide insufficient stimulation resulting in abortive T cell proliferation, «active» DCs in the early phase of the immune response provide optimal conditions for driving differentiation of effector T cells and «exhausted» DCs at later time points may give just the right amount of stimulus required to prime T cells without inducing their differentiation. Experiments are ongoing to test this hypothesis and to identify the optimal conditions for the generation of memory cell precursors capable of generating T_{CM} and T_{EM} when antigen is cleared.

Flexible programs of gene expression in human polarized T lymphocytes

Mara Messi and Federica Sallusto

We have previously shown that human polarized T_H1 and T_H2 cells, when restimulated in the presence of IL 4 and IL 12, respectively, acquire the capacity to produce the opposite cytokine while retaining the expression of the originally imprinted cytokine IFN-γ or IL 4. These findings indicate that T cells maintain memory of the initial polarization but have the flexibility to undergo additional differentiation programs. We asked whether the flexibility of cytokine gene expression would apply to tissue T cells involved in an inflammatory immune response *in vivo* and whether, besides cytokine genes, other T_H1- or T_H2-related genes may be induced in polarized T cells. To address the first question we analyzed different T cell populations that were distinguished by the expression of chemokine receptors infiltrating the synovia of adult rheumatoid patients or the skin of atopic dermatis patients. All the subsets analyzed, derived either from synovia or skin, beard a more or less pronounced degree of flexibility, being able to acquire IFN-γ or IL-4 production when stimulated under appropriate polarizing condition. The analysis also confirmed our previous results indicating that effector memory CRTh2$^+$ cells have a fixed phenotype. To address the second question we are performing Affymetrix analysis on T_H1 and T_H2 clones that have been recloned under homologous or opposite polarizing condition. Our preliminary results show that, while IL-4 production is retained in T_H2 cells stimulated in T_H1-condition, the expression of T_H2-associated chemokine receptors is lost whereas the T_H1-associated chemokine receptors are acquired. These results indicate that cytokine and chemokine receptor genes are regulated by different mechanism and provide a plausible explanation for the observed dissociation between expression of cytokines and chemokine receptors found in a small proportion of memory T cells. This work has been initiated by Stephane Chappaz and is done in collaboration with Francesco Bertoni and Andrea Rinaldi, IOSI, Bellinzona, Switzerland.

Nuclear localization of TH1- and TH2-specific genes in human memory T lymphocytes

Mara Messi and Federica Sallusto

When stimulated *in vitro* under polarizing conditions mouse CD4$^+$ T cells become rapidly committed to the T_H1 or T_H2 lineage. This process is accompanied by repositioning of the silenced cytokine gene to heterochromatic regions. In contrast, most human T_H1 and T_H2 cells are not irreversibly committed since the non-expressed cytokine gene can become, under appropriate stimulatory condition, accessible to the transcriptional machinery. We previously identified a subset of human memory T cells that is irreversibly committed to the T_H2 lineage. These cells did not express the T_H1-specifying transcription factor T-bet nor upregulate it upon TCR stimulation. Transfection of a plasmid encoding T-bet in these cells confers the capacity to express IFN-γ, suggesting that irreversible commitment may require silencing of the cell fate-determining transcription factors. Chromatin immunoprecipitation experiments demonstrated association of *Tbet* promoter with deacetylated histones in committed T_H2 cells but not in naïve and T_H1 cells. However, in resting and activated T_H2 cells the majority of *Tbet* alleles (90%) as well *Ifng* alleles (63%) were localized away from silenced centromeric chromatin domains as assessed by fluorescence *in situ* hybridization (FISH) using an α-satellite-specific probe. These results indicate that in human memory T lymphocytes repositioning to heterochromatin is not required for irreversible silencing of lineage-specific genes.

In collaboration with Susannah Hewitt and Matthias Merkenschlager, Imperial College London, London, UK.

Dynamics of antigen specific CD4 T cells within memory subsets studied by repertoire analysis

Jens Geginat, Antonio Lanzavecchia,
and Federica Sallusto

Memory T cells can be divided into follicular helper (T_{FH}) central (T_{CM}) and effector (T_{EM}) memory subsets with distinct functions and homing capabilities. We are analyzing the composition and dynamics of CD4$^+$ tetanus-toxoid (TT) specific T cells in these memory populations at different time points after vaccination. In order to obtain antigen specific CD4$^+$ T cells we developed a CFSE-based assay that allowed the efficient isolation of T cells that proliferated in response to antigenic stimulation. This method can be used for any antigen and HLA combination, is sensitive and allows estimation of frequencies of specific T cells present in the starting populations. Furthermore, it allows the accurate removal of non-proliferating cells and the generation of pure preparations of antigen specific T cells for molecular analysis. Using this methodology we measured the overall complexity of the TCR repertoire of T_{FH}, T_{CM} and T_{EM}. The results obtained so far by Vβ-Cβ immunoscope analysis indicate that the response to TT involves a large number of clonotypes, this number being higher in T_{CM} (124 and 73 peaks in donor 1 and 2, respectively) than T_{EM} (93 and 70) and T_{FH} (76 and 66). We are now performing extensive sequencing analysis to identify clonotypes in different memory subsets and at different time points.

In collaboration with Cécile Bouneaud, Laurent Ferradini and Christophe Pannetier, INSERM U277, Paris, France and Paolo Dellabona and Giulia Casorati, Dibit-H. S.Raffaele, Milano, Italy.

Coexpression of CD25 and CD27 identifies FoxP3$^+$ regulatory T cells in inflamed synovia

Claudia R. Ruprecht, Antonio Lanzavecchia, and Federica Sallusto

There is now clear evidence that a distinct population of naturally occurring regulatory T cells which can be identified by the constitutive expression of CD4 and CD25 play an essential role in controlling autoimmunity. However the identification of regulatory T cells in an ongoing immune response or in inflamed tissues is complicated by the fact that CD25 is expressed also by activated conventional T cells. We found that the CD4$^+$CD25$^+$ population in synovial fluid of juvenile idiopathic arthritis (JIA) patients comprises both regulatory and effector T cells that can be distinguished by expression of CD27. CD4$^+$CD25$^+$CD27$^+$ cells expressed high amounts of FoxP3 (43% of them being FoxP3$^+$), did not produce IL-2, IFN-γ or TNF, and suppressed T cell proliferation *in vitro* being, on a per cell basis, 4-fold more potent than the corresponding peripheral blood population. In contrast, CD4$^+$CD25$^+$CD27$^-$ cells expressed low amounts of FoxP3, produced effector cytokines and did not suppress T cell proliferation. Following *in vitro* activation and expansion regulatory but not conventional T cells maintained high expression of CD27. IL-7 and IL-15 were found to be present in synovial fluid of JIA patients and when added *in vitro* abrogated the suppressive activity of regulatory T cells. Taken together these results demonstrate that, when used in conjunction with CD25, CD27 is a useful marker to distinguish regulatory from effector T cells in inflamed tissues and suggest that at these sites IL-7 and IL-15 may interfere with regulatory T cell function.

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Immune Regulation

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Introduction

Encounter of naïve T cells with antigen-carrying dendritic cells (DCs) leads either to T cell deletion and tolerance or to T cell proliferation and generation of effector and memory cells. There is growing evidence that innate immunity controls DC activation that directly impacts on the adaptive immune response. A first goal of our laboratory is to understand how microbial products, inflammatory cytokines and T cells regulate DC maturation and function. A second and related goal is to understand how T cell fate is determined. Using several experimental systems we are testing the hypothesis that T cell fate is a function of the cumulative strength of stimulation (SoS) received by naïve T cells.

Once appropriately primed memory T and B cells can be maintained for a lifetime. A «central» compartment of memory T and B cells contains differentiation intermediates and mediates secondary responses, while and «effector» compartment contains terminally differentiated T and plasma cells and is involved in immediate protection. We are interested to understand the mechanisms that control the generation of T and B memory cells and the dynamics of memory cells in the central and effector compartment. In particular we aim at identifying memory cells with self renewal capacity (i.e. the memory stem cells).

The above studies are expected to translate into: i) novel adjuvants capable of driving stronger or selected immune responses; ii) *in vitro* correlates of the immune status to evaluate vaccine efficacy and iii) adoptive immunotherapies with T cells or antibodies retrieved from the memory repertoire.

Toll like receptors at the interface between innate and adaptive immunity

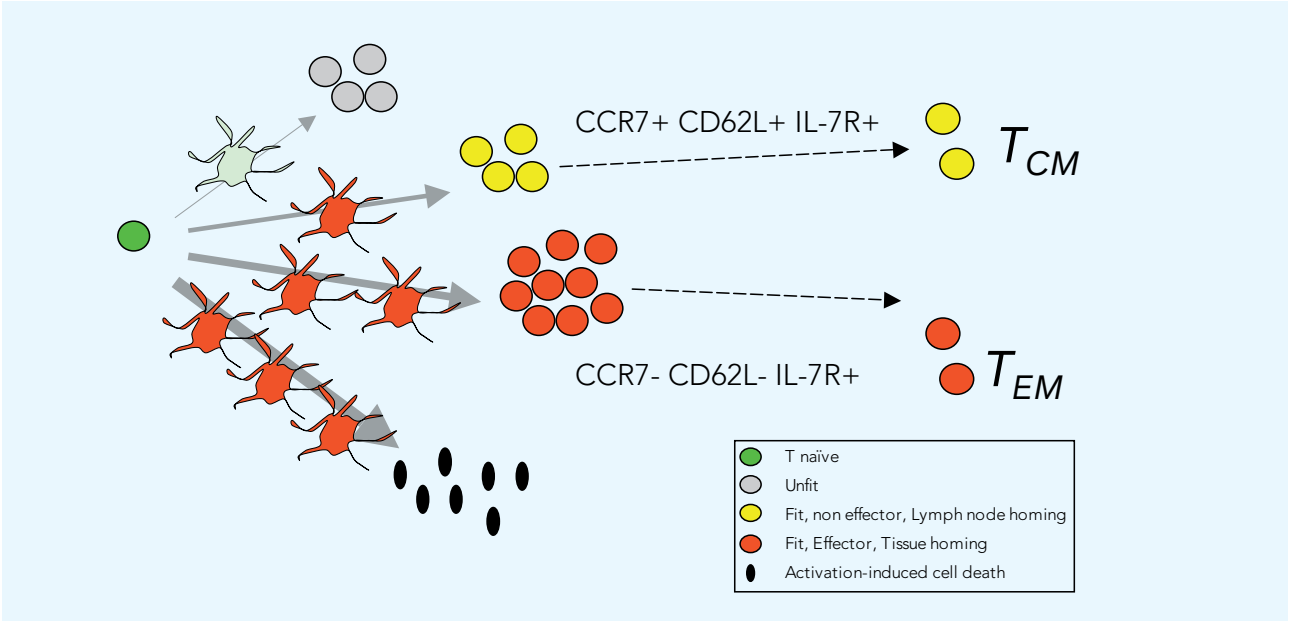
Since the original description of a method to generate human immature DCs (Sallusto & Lanzavecchia, 1994), our laboratory has used this system to identify the stimuli that induce DC maturation. Initial studies showed that maturation could be triggered by microbial products (such as LPS and polyIC), by inflammatory cytokines (TNF and IL-1) and by T cells (through CD40L) (Sallusto & Lanzavecchia, 1994; Sallusto et al., 1995; Cella et al., 1996; Cella et al., 1997; Cella et al., 1999; Gagliardi et al., 2000). Recently, with the discovery of the members of the TLR family and their specificity for microbial products, our work has focused on the analysis of the distribution and function of TLRs in human DC subsets and in B cells. These studies revealed that human monocyte-derived DCs and interferon producing cells (IPCs) express complementary sets of TLRs and therefore respond to different microbial products (Jarrossay et al., 2001). TLRs were found to be constitutively expressed in memory, but not in naïve B cells and were rapidly upregulated in naïve B cells upon BCR stimulation (Bernasconi et al., 2002; Bernasconi et al., 2003). The presence of several TLRs in the same cells begs the question of whether these receptors may synergize among themselves or with other triggering receptors in promoting DC and B cell activation.

Strength of stimulation and T cell differentiation: the SoS model

Our interest in this field stems from two original observations, namely that: i) TCR signaling needs to be sustained for several hours (through a process of serial triggering) in order for the T cell to become committed to proliferation and differentiation (Valitutti et al., 1995; Iezzi et al., 1998) and ii) memory T cells comprise two subsets with distinct homing capacity and effector function, central memory T cells (T_{CM}) and effector memory T cells (T_{EM}) (Sallusto et al., 1999). Further studies from our laboratory clarified the contribution of costimulation and cytokines in determining the extent and quality of T cell differentiation (Lanzavecchia et al., 1999; Viola et al., 1999). To explain the generation of different types of memory T cells we hypothesized that T cell differentiation is determined by the cumulative strength of stimulation (SoS), i.e. the intensity and duration of TCR and cytokine signal (Lanzavecchia & Sallusto, 2000; Lanzavecchia & Sallusto, 2001; Sallusto et al., 2004). As a function of the SoS received naïve T cells progress through hierarchical thresholds for proliferation, survival, switch of homing receptors and acquisition of effector functions (Iezzi et al., 1998; Iezzi et al., 2001; Langenkamp et al., 2000). The stochastic process of T cell stimulation via random interactions with DCs results in the generation of various intermediates and terminally differentiated effector cells, which are then selected as memory cells according to their survival and homing capacity (defined as «fitness»). This model has been extensively validated on human T cells *in vitro* (Langenkamp et al., 2000; Langenkamp et al., 2002). *In vivo* we have shown that mouse T cells primed with limiting SoS home to lymph nodes and persist as central memory T cells capable of generating secondary response, while cells primed with higher SoS become effectors and home to peripheral inflamed tissues (Iezzi et al., 2001). We have subsequently shown that by further limiting SoS it is possible to expand T cells that are incapable of surviving since they fail to upregulate anti-apoptotic molecules and receptors for homeostatic cytokines (Gett et al., 2003).

Homeostasis of memory T and B cells: the memory stem cell model

Once generated by appropriate priming the «central» and «effector» compartments can be maintained for a lifetime in the absence of further antigenic stimulation. We hypothesized that in secondary lymphoid organs T_{CM} and memory B cells proliferate and differentiate to effector T cells and plasma cells in response to antigen independent stimuli such as homeostatic cytokines or bystander T cell help. This «stem cell model» of memory cell maintenance implies that the central memory cells are capable of self renewal and of replenishing a compartment of terminally differentiated cells, very much like hematopoietic stem cells do (Lanzavecchia & Sallusto, 2000; Sallusto et al., 2004). This model is supported by the finding that human memory $CD4^+$ T cells (Geginat et al., 2001) and $CD8^+$ T cells (Geginat et al., 2003) proliferate and differentiate spontaneously in response to homeostatic cytokines such as IL-7 and IL-15 and that memory B cells proliferate and differentiate to plasma cells in response to TLR agonists or bystander T cell help (Bernasconi et al., 2002).



T cell fate determined by strength of stimulation (as depicted by arrow size)

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Ongoing projects

Selected TLR agonist combinations synergistically trigger DCs with enhanced T_H1 polarizing capacity

Giorgio Napolitani, Federica Sallusto,
and Antonio Lanzavecchia

TLRs sense microbial products and initiate adaptive immune responses by activating DCs. Since pathogens may contain several agonists, we asked whether different TLRs may synergize in DC activation. We found that in human and mouse DCs TLR3 or TLR4 potently synergize with TLR7, TLR8 or TLR9 in the induction of selected cytokine genes. Upon synergistic stimulation, IL-12, IL-23 and Delta-4 were induced at levels 50-100 fold higher than those induced by optimal concentrations of single agonists, leading to enhanced and sustained T_H1 polarizing capacity. Using microarray analysis we found that only 1.5% of the transcripts induced by single TLR agonists were synergistically regulated by combinations of TLR4 and TLR8 agonists. These results identify a combinatorial security code by which DCs discriminate pathogens and provide a rationale to design adjuvants for T_H1 responses. This work was done in collaboration with Francesco Bertoni and Andrea Rinaldi, IOSI, Bellinzona, Switzerland.

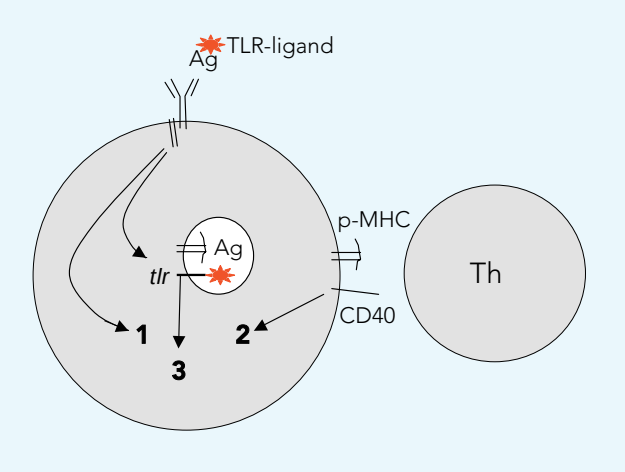

Figure 1. Three signals are necessary for B cell activation.

Sustained TLR stimulation is required for transcription of late genes in maturing DCs

Giorgio Napolitani and Antonio Lanzavecchia

Synergic TLR stimulation leads to increased expression of a selected set of genes (primarily late genes) involved in priming T_H1 and inflammatory T cells. We found that synergistic TLR stimulation sustained phosphorylation of c-Jun at late time points coincident with transcription of late genes (8-12 hours). Remarkably, removal of TLR agonists at early time points (1-5 hours) did not interfere with DC maturation as assessed by upregulation of MHC and costimulatory molecules, but abolished c-Jun phosphorylation and late gene transcription. These findings indicate that DCs can sense the duration of exposure to microbial stimuli and that a sustained stimulation of TLRs is required for elicitation of late transcripts. These results also imply that continual TLR engagement by microbial products can sustain signal and control transcription.

A role for plasmacytoid DCs in differentiation of follicular helper T cells and plasma cells

David Jarrossay and Antonio Lanzavecchia

CXCL13 is a chemokine produced in B cell areas that attracts $CXCR5^+$ T and B cells. CXCR5 is expressed on a small subset of $CCR7^-$ T cells in B cell follicles (therefore defined follicular helper T cells, T_{FH}) and on a subset of circulating T_{CM}. We found that plasmacytoid DCs stimulated by influenza virus (or CpG) and T cells (or soluble CD40L and TNF) upregulate CXCR5 and produce CXCL13, IFN-I and IL-6. When cultured with these mature pDCs circulating $CCR7^+CXCR5^+$ T_{CM} readily differentiate to T_{FH} by losing CCR7 and producing high levels of IL-10. In tonsils pDCs localize to the border of B cell follicles together with $CXCR5^+$ T and B cells. These findings suggest that following viral priming and T cell licensing, pDCs localize at the border of T cell areas where they can interact with $CXCR5^+$ T cells that enter the follicle and with antigen-activated B cells that exit the follicle. In this way activated pDCs may promote differentiation of T_{FH} and their encounter with activated B cells.

Essential role for TLRs in the activation of human naïve B cells

Claudia Ruprecht and Antonio Lanzavecchia

It is generally accepted that B cell priming requires two signals delivered by antigen and helper T cells. Using an improved method to isolate human naive B cells we investigated the minimal requirements for their expansion and differentiation *in vitro*. We found that BCR stimulation plus cognate T cell help triggered initial proliferation of naïve B cells, but were not sufficient to promote their accumulation. Extensive B cell proliferation, isotypic switch and differentiation to Ig-secreting cells required a third signal that could be delivered by triggering TLR2, TLR6, TLR7 and TLR9, which were upregulated in human naïve B cells upon BCR stimulation. In addition, DCs triggered through TLR3 and TLR4 (which are not expressed by B cells) were able, at least in part, to provide the third signal via production of IL-12 and IL-6. We conclude that microbial products acting directly on B cells (or indirectly through DCs) provide an essential third signal for activation of human naïve B cells (Figure 1).

T cell differentiation and memory generation as a function of strength of stimulation (SoS)

Greta Guarda, Jens Geginat, Federica Sallusto, and Antonio Lanzavecchia

To understand which signals are required for T cell priming and for the generation of memory T cells, we set up an experimental system in which mouse TCR transgenic $CD4^+$ and $CD8^+$ T cells are stimulated *in vitro* by mature DCs and the SoS delivered is varied as a function of the T/DC ratio and the duration of DC – T cell interaction. We found that L-selectin and CCR7 expression, as well as *in vivo* homing to the lymph nodes decreased progressively at increasing SoS, whereas responsiveness to homeostatic cytokines increased. Furthermore, the ability of the activated T cells to mount a secondary response *in vitro* and *in vivo* decreased as SoS increased. We conclude that for both $CD4^+$ and $CD8^+$ T cells memory generation requires an intermediate SoS, that is sufficient to make T cells responsive to homeostatic cytokines, while preserving their ability to home to secondary lymphoid organs and to respond to secondary antigenic challenge.

Regulation of IL-7R expression and IL-7 responsiveness in activated human T cells

Laura Rivino, Greta Guarda, David Jarossay, Federica Sallusto, Antonio Lanzavecchia, and Jens Geginat

Recently it was shown that in mice IL-7 is required for

memory cell generation and that early memory cell precursors express the α-chain of the IL-7 receptor (IL-7Rα). We analyzed IL-7Rα expression and IL-7 responsiveness in human CD4$^+$ naïve T cells that were primed by different strengths of stimulation (SoS). We found that IL-7Rα is rapidly lost upon T cell activation and is regained at later time points in different proportions depending on SoS. At low and high SoS, only few T cells express IL-7Rα, but fail to proliferate since in the first case the receptor did not signal, while in the second the cells responded but showed high death rate. In contrast at intermediate SoS a large proportion of T cells was IL-7Rα$^+$ and these cells responded to IL-7 by STAT5 phosphorylation and activation of the p70s6 kinase. These cells were heterogeneous as far as CCR7 expression and proliferation and differentiation potential in response to IL-7α Some, nonpolarized CCR7$^+$, maintained this phenotype while proliferating in response to IL-7. Others were CCR7$^+$ but upon IL-7 driven proliferation lost CCR7 and spontaneously differentiated to T_H1. Finally CCR7$^-$ cells upon culture in IL-7 reacquired CCR7 expression, but failed to accumulate due to high death rate. These *in vitro* findings suggest that memory cell generation requires an intermediate SoS and that non-polarized T_{CM}, pre-T_{EM} and T_{EM} might be generated from heterogeneous CCR7$^+$IL-7Rα$^+$ precursors.

A CCR7 suicide reporter mouse to study the role of mature DCs and of central memory T cells in vivo

Nobuyuki Onai, Aja Onai, Greta Guarda, Federica Sallusto, and Antonio Lanzavecchia

To test *in vivo* the role of CCR7$^+$ mature DCs and central memory T cells (TCM) we used the BAC technology to produce transgenic mice in which the CCR7 promoter drives monkey diphtheria toxin (DT) receptor expression. The mice are viable and breed normally. Injection of DT *in vivo* led to rapid depletion of naïve T and B cells but not CD11c$^+$, CD11b$^+$ cells or NK cells, consistent with the pattern of CCR7 expression. *In vitro* experiments indicate that non-polarized cells capable of proliferating in response to antigenic stimulation can be selectively deleted by DT while effector T cells are spared. Furthermore, mature transgenic DCs injected in allogeneic hosts can be selectively killed by DT resulting in a >90% inhibition of the T cell response *in vivo*. After extensive backcross this model should be suitable to address the role of T_{CM} cells using an adoptive transfer system.

Characterization of IL-10 producing T cells

Laura Rivino, David Jarossay, Federica Sallusto, Antonio Lanzavecchia, and Jens Geginat

Peripheral tolerance can be achieved by several mechanisms including deletion of activated T cells or suppression by specialized T cell populations. Two major regulatory T cell subsets have been described: i) CD25$^+$ «natural» Tregs which are generated in the thymus and express the transcription factor FoxP3 and ii) «adaptive» Tregs, that are generated in the periphery under tolerogenic priming conditions from naïve T cells and secrete suppressive cytokines IL-10 and/or TGF-β. No surface markers for human adaptive Tregs have been identified so far. We monitored chemokine receptor modulation upon tolerogenic versus immunogenic T cell priming. We found that tolerogenic conditions and TGF-β promote the generation of cells expressing CCR4 and CCR6. CCR4$^+$CCR6$^+$ cells are enriched among CD25$^+$ Tregs, but are also detectable among antigen-experienced CD25$^-$CD4$^+$ T cells that do not express FoxP3. The latter secrete high amounts of IL-10 but other cytokines. Furthermore, CCR4$^+$CCR6$^+$ T cells proliferate in response to autologous myeloid DCs in the presence of neutralizing IL-10 and/or TGF-β antibody in a MHC class II-restricted manner. Interestingly, CCR4$^-$CCR6$^+$ T cells also show auto-reactivity and secrete IL-10, but they also produce high levels of IFN-γ. The suppressive potential and the responses to antigens involved in autoimmune diseases of CCR6$^+$ subsets are under current investigation.

Segregations and overlaps in the homeostatic control of non-naïve T cells

Afonso Almeida and Antonio Lanzavecchia

How the immune system maintains each of the relevant memory subpopulations and how independent is their homeostatic regulation is largely unknown. We will investigate if the memory T cell pool relies on the segregation of the homeostatic control of its sub-populations (e.g. T_{CM} and T_{EM}) or if these subpopulations belong to a common pool and may thus be co-regulated. We will also investigate the impact of lymphopenia-driven proliferation and bystander proliferation on these sub-populations and study if cells generated via these pathways compete for common peripheral T cell pools with true memory T cells. We will use TCR transgenic and wild type mice expressing different allotypic markers in competitive repopulation studies. In parallel, we will also study the repopulation abilities of human cells undergoing lymphopenia-driven proliferation, taking advantage of the AIS mouse model recently developed by Markus Manz and coworkers. We will transfer human T cells generated in these mice into secondary lymphopenic recipients and study the reconstitution of specific sub-populations of memory T cells, which have been described in humans in greater details than in mice. In the first months, the work has been centered in the breeding and expansion of the different mouse strains required.

Mechanisms that sustain serum antibody levels following vaccination

Elisabetta Traggiai and Antonio Lanzavecchia

Following vaccination or infection constant levels of specific antibodies can be maintained in human for a lifetime. This phenomenon requires sustained antibody production since the half-life of the IgG antibodies is only 3 weeks. Long-lived plasma cells resident in the bone marrow and memory B cells, stimulated to differentiate to plasma cells by persisting antigen or by polyclonal stimulation, may contribute to enduring antibody responses. However, their relative contribution to a continuous antibody production remains to be established. We monitored for two years the kinetics of circulating plasma cells, memory B cells and serum antibodies in 8 healthy volunteers that had been boosted with tetanus toxoid (TT). In all cases we observed a rapid elevation of serum antibodies (10-100 fold increase from day 6 to day 10) followed by a plateau lasting about a month, which was followed by a linear decrease in antibody levels until a stable plateau value was reached after 6-8 months. This stable value was higher than the pre-boost value and the increase correlated with an increased frequency of memory B cells. The experimental values obtained have been used to elaborate and validate a mathematical model (developed in collaboration with Roberto Puzone, University of Genova). The equation models the kinetics of serum antibodies on the basis of three parameters: i) magnitude of the antigen driven short lived plasma cell response (responsible for the rapid elevation of serum antibodies); ii) magnitude of the antigen driven long lived plasma cell response (that sustain serum antibodies for a few months) and iii) extent of homeostatic proliferation and differentiation of memory B cells (that sustains antibody production after the antigen dependent phase). Based on these results and their modeling (Figure 2) we propose two memory phases: a short term memory, which is determined by short lived and long lived plasma cells generated following antigenic stimulation, and a long term memory that is maintained through antigen independent polyclonal activation of memory B cells. The model of serological response generated may be used to predict the effect of vaccination.

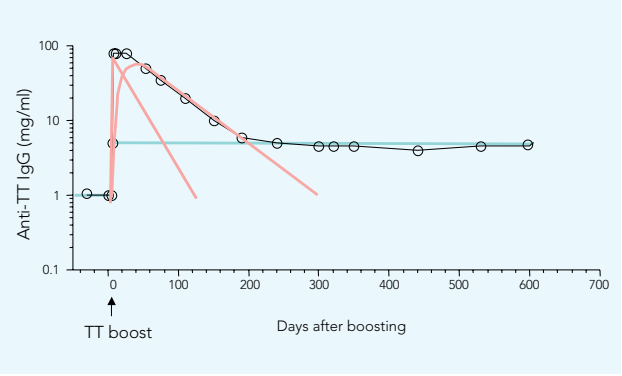

Figure 2. Modelling the serological response. Time course of serum antibody levels (circles) in a volunteer boosted with tetanus toxoid; contribution of antigen induced short-lived and long-lived plasma cells (red lines); contributions of antigen independent polyclonal activation (blue lines).

ABCB1 transporter discriminates human resting naive B cells from cycling transitional and memory B cells

Stefan Wirths and Antonio Lanzavecchia

The exact identification of B cell subsets is instrumental to understand their dynamics under physiological and pathological conditions. Human memory B cells are currently identified according to the expression of CD27 which is absent on naïve B cells. We found that the ABCB1 transporter is exclusively present on mature $CD27^-$ naïve B cells, while it is absent in $CD27^+$ memory B cells and in a heterogeneous subset of $CD27^-$ cells that comprise both switch memory and transitional B cells. Thus ABCB1 activity precisely discriminates naïve from transitional and all memory B cells. Using this improved method to discriminate human B cell subsets and Ki67 staining to identify recently divided cells, we found that in both cord blood and adult peripheral blood mature naïve B cells are quiescent, while transitional B cells and memory B cells have high *in vivo* turnover.

An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS-coronavirus

Elisabetta Traggiai and Antonio Lanzavecchia

Passive serotherapy can confer immediate protection against several microbial infections, but methods to rapidly generate human neutralizing monoclonal antibodies are not yet available. We developed an improved method of EBV transformation of human B cells and used this method to analyze the memory repertoire of a patient recovered from SARS and to isolate several neutralizing and non-neutralizing monoclonal antibodies (Figure 3). One such antibody specific for the SARS coronavirus (SARS-CoV) spike protein has potent *in vitro* neutralizing activity and confers protection *in vivo* in a mouse model of SARS-CoV infection. These results show that in case of an emerging disease it is possible to interrogate the memory repertoire of immune donors to rapidly and efficiently isolate neutralizing antibodies which have been selected in the course of natural infection. This work was done in collaboration with Stephan Becker, University of Marburg and Kanta Subbarao, NIAID, Bethesda and Gary Nabel, VRI, Bethesda.

Neutralization of bacterial toxins by human monoclonal antibodies

Davide Corti, Nadia Bernasconi,
and Antonio Lanzavecchia

Polyclonal antibodies to bacterial toxins have been used as life-saving drugs for more than a century. However there are still no human antibodies available to neutralize Diphteria or Anthrax toxins in exposed individuals. By immortalizing memory B cells from an immunized volunteer we were able to screen 165 monoclonal antibodies that reacted in ELISA with Anthrax toxin protective antigen (PA). Only a fraction of the antibodies neutralized the Anthrax holotoxin (PA+LF) *in vitro*, while others failed to neutralize or showed a marked prozone. The most effective antibody (A146) completely neutralized Anthrax holotoxin at substoichiometric concentrations. Furthermore, 30 µg of A146 protected rats from a lethal challenge with 80 µg holotoxin. In another setting, individuals with high antibody titers to Diphteria toxin (DT) were identified and several monoclonal antibodies were isolated from one of thiese individuals. The most effective antibody (D2.2) neutralized DT *in vitro* in a stoichiometric fashion at concentrations as low as 10^{-11}M. Other monoclonal antibodies showed lower potency and only partial neutralization. We conclude that highly potent antibodies capable of neutralizing bacterial toxins can be selected from the memory repertoire of immune donors. This work is done in collaboration with Bob Mittler, Emory University and Cesare Montecucco, University of Padova.

The complexity of the B cell response to malaria and HIV

Nadia Bernasconi, Davide Corti,
and Antonio Lanzavecchia

The composition and stability of memory B cell pool specific for chronic persistent and highly variable infectious agents such as Plasmodium falciparum or HIV is largely unexplored. We are interrogating the memory B cell repertoire of multiparous African women infected with P. falciparum to isolate B cells producing antibodies that recognize i) the variant antigens involved in the sequestration of infected erythrocytes in placenta or brain; ii) the merozoite surface protein-1 (MSP-1) involved in invasion of red blood cells and iii) a lysate of infected red blood cells. The results obtained so far indicate that a large fraction of memory B cells is specific for P. falciparum antigens and that a small fraction of this repertoire is directed against variant antigen and MSP-1 peptides. Several monoclonal antibodies have been isolated and will be tested in functional assays and in animal models for their capacity to block erythrocyte invasion and sequestration of infected erythrocytes. We have also undertaken a clonal analysis of memory B cells in HIV patients with the aim of establishing the immunodominance of different antigens and of isolating broadly neutraizing antibodies. This work is done in collaboration with Lea Barfod and Lars Hviid, Rigshospitalet, Copenhagen, Hermann Bujard, ZMBH, University of Heidelberg, and Pascal Poignard, CIML, Marseille.

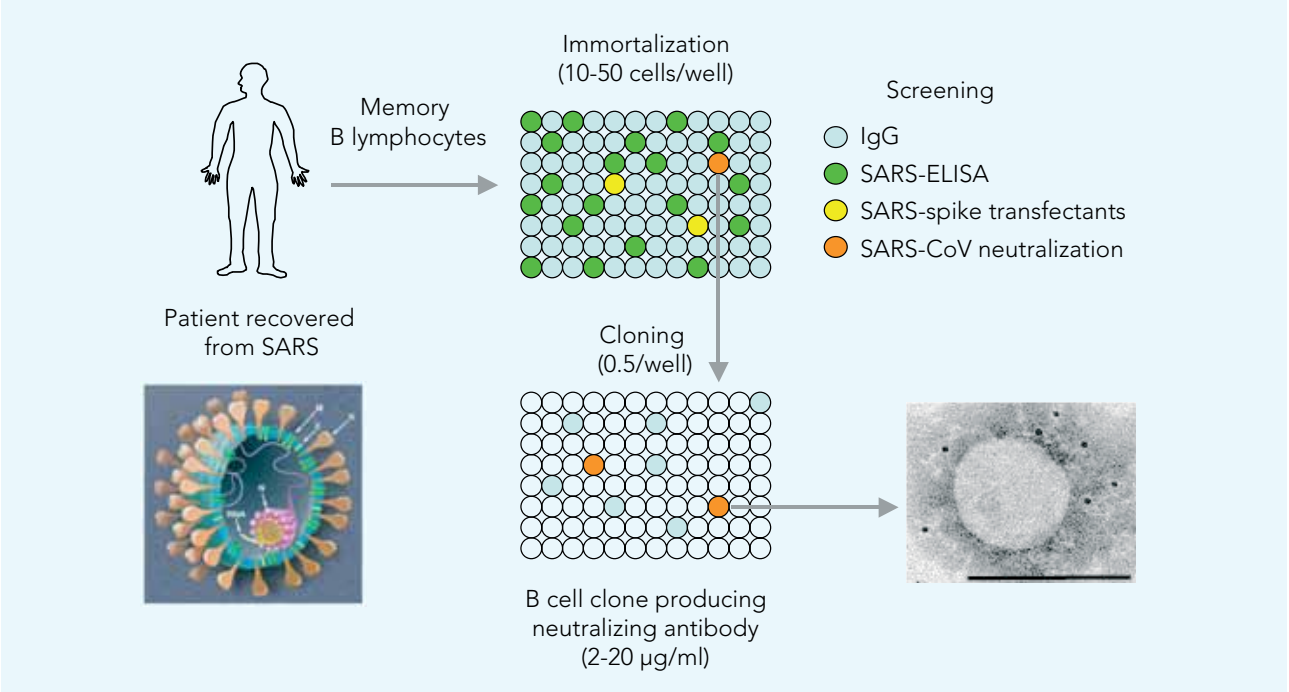

Figure 3. Isolation of human monoclonal antibodies against SARS Corona virus from an immune donor.

Beneficial autoantibodies in cancer, autoimmunity and neurodegenerative diseases

Elisabetta Traggiai, Nadia Bernasconi,
Barbara Paviglianiti, Mariagrazia Uguccioni,
and Antonio Lanzavecchia

Autoantibodies are produced in a variety of situations ranging from healthy donors to patients with autoimmune diseases or cancer. While autoantibodies reacting against intracellular antigens may be devoid of pathogenetic or therapeutic effects, those that react with secreted or surface molecules may be responsible for significant pathology or, in some instances, even protection. Indeed, there is convincing evidence that some autoantibodies may be beneficial, for instance the antibodies to inflammatory cytokines found in patients with autoimmune diseases and antibodies to A-beta peptides in Alzheimer patients. By using the improved EBV-immortalization method we have undertaken a systematic effort to isolate such beneficial autoantibodies from selected patients with melanoma, rheumatoid arthritis, Sjogren syndrome or Alzheimer. This work is done in collaboration with Alex Knuth and Roger Nitsch University of Zurich, Switzerland and Carlo Montecucco, University of Pavia, Italy.

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PhD Programme

The IRB provides high level scientific education for graduate students. The PhD programme, carried out in collaboration with Swiss and foreign universities, includes experimental work carried out at IRB under the direct supervision of a group leader as well as seminars, lessons, and an annual PhD student retreat. Starting from 2004 the Institute organizes an International PhD lecture course that includes lectures and journal clubs. In addition, the Istitute participates to an international PhD programme coordinated by the Vita Salute San Raffaele University of Milan, Italy. The IRB also provides education for undergraduate students (short stages and experimental diploma thesis).

PhD Thesis

«The critical role of dendritic cells for T cell priming and differentiation»

Anja Langenkamp

University of Witten/Herdecke, Germany – 2002

Dr. Anja Langenkamp from Neviges (Germany) studied and graduated in biochemistry at the University Witten/Herdeke (Germany). In 1999 she started to work on her PhD thesis in the group of Prof. Antonio Lanzavecchia and Dr. Federica Sallusto at the Basel Institute of Immunology. In April 2000 when the IRB opened his doors for scientific work she moved to Bellinzona. In June 2002 Anja Langenkamp successfully defended her thesis on «The critical role of dendritic cells for T cell priming and differentiation» at the University of Witten/-Herdeke, under the co-supervision of Prof. K.S. Zänker. In her thesis Anja Langenkamp provides new information on the regulation of T cell responses by dendritic cells. She shows the effects of microbial products and adjuvants for the maturation of human dendritic cells. A chapter of her thesis describes the precise kinetics of cytokine production by dendritic cells and the consequences for T cell polarization. She also identifies parameters that influence the efficiency of T cell priming and differentiation and investigates the distinct kinetics of chemokine receptor expression on differentiating T cells. The studies are published in renowned journals: *Eur. J. Immunol. (2000) 30:2394-403; Curr. Top. Microbiol. Immunol. (2000) 251:167-71; Nat. Immunol. (2000) 1:311-6 and Eur. J. Immunol. (2002) 32:2046-54.*

«Regulation of cytokine gene expression in human effector and memory T lymphocytes»

Mara Messi

University of Bern, Switzerland – 2003

Dr. Mara Messi graduated in Molecular Biology at the University of Zurich. In April 2000 she entered the PhD program of the IRB in the group of Dr. Federica Sallusto and in July 2003 she defended her thesis on the «Regulation of cytokine gene expression in human effector and memory T lymphocytes» at the University of Bern, under the co-supervision of Prof. Hans-Uwe Simon.

Mara Messi investigated the stability and flexibility of cytokine gene expression in human T helper (Th) cells. She discovered that effector memory Th1 and Th2 cells in vivo contain acetylated histones associated with the IFN-γ and IL-4 gene promoters, respectively. This epigenetic modification contributes to confer and possibly maintain the memory for cytokine production through cell divisions. Furthermore, she found that human T cells display a high degree of flexibility that is not shared by mouse T cells. This study is published in *Nat. Immunol. (2003) 4:78-86.*

She also investigated the regulation of cytokine gene expression in activated human T cells involved in ongoing chronic inflammatory responses in vivo (rheumatoid arthritis, atopic dermatitis). This study confirmed previous findings and provided new information on the molecular and cellular aspects of T cell-mediated immunopathologies.

Nadia Bernasconi

«Selective response of human memory B cells to polyclonal activators: a mechanism maintaining serological memory»

University of Fribourg, Switzerland – 2003

Dr. Nadia Bernasconi graduated in Biochemistry and Molecular Biology at the University of Southern California in Los Angeles. In August 2000 she entered the PhD program of the IRB in the group of Prof. Antonio Lanzavecchia and in July 2003 she defended her thesis on the «Selective response of human memory B cells to polyclonal activators: a mechanism maintaining serological memory» at the University of Fribourg, Switzerland, under the co-supervision of Prof. Sandro Rusconi.

Nadia Bernasconi studied the requirements for proliferation and differentiation of human naïve, IgM^+ memory and switch memory B cells. She found that, in contrast to naïve B cells, which are dependent on BCR signaling, memory B cells can be selectively activated by polyclonal stimuli such as CpG, cytokines or T cell help, in the absence of antigen. Based on these findings she considered the possibility that a continuous polyclonal activation of memory B lymphocytes may sustain plasma cell generation and antibody production resulting in long term serological memory. The results were published in *Science (2002) 298:2199-2202*.

In another chapter of her thesis she investigated the role of Toll like receptors (TLRs) in human B cell activation. She found that in human naïve B cells, most TLRs are expressed at low to undetectable levels, but the expression of TLR9 and TLR10 is rapidly induced following B-cell-receptor (BCR) triggering. In contrast, memory B cells express several TLRs at constitutively high levels. The requirement of BCR to induce expression of TLRs in human naïve B cells prevents polyclonal activation in a primary response and restricts the stimulation to antigen-specific B cells. The constitutive expression of TLRs in memory B cells allows a polyclonal activation of the entire memory pool. Thus, in human B cells TLRs are downstream of BCR and play a role both in the primary response and in the memory phase. Published in *Blood 2003:101:4500-4504*.

In 2002, together with Elisabetta Traggiai, she received the ASIRB – Roche Research Award for the work on «Polyclonal activation of human memory B cells and maintenance of serological memory».

«Study on the T cell reactivity against myelin basic protein in Multiple Sclerosis patients»

Elisabetta Traggiai

University of Florence, Italy – 2003

Dr. Elisabetta Traggiai graduated in Biology at the University of Pisa. She started in April 2000 her doctorate at the Department of Neurological Science of the University of Florence, under the supervision of Prof. Domenico Inzitari. In June 2003 she defended her thesis on the Characterization and regulation of T and B autoreactivity to myelin basic protein (MBP) in Multiple Sclerosis patients. In November 2001 Elisabetta Traggiai joined the group of Prof. Antonio Lanzavecchia at the IRB where she investigated the mechanisms involved in secondary antibody responses and in the maintenance of long-term serological memory. She tested the hypothesis that memory, but not naïve B cells, are continuously activated to proliferate and differentiate into plasma cells *in vitro* and *in vivo* in response to polyclonal stimuli, such as bystander T cell help or Toll-Like Receptor (TLR) agonists, thus maintaining a constant antibody production in the absence of antigen. The results of this study were published in *Science (2003) 298: 2199-2202*. She performed a quantitative analysis of the secondary immune response to a thymus dependent antigen to reveal the relative contribution of antigen induced short- and long- lived plasma cells versus polyclonal activation of all memory B cells. Antigenic boost sustains high levels of serum antibodies only for a few months, while polyclonal activation sustains low levels of protective antibodies for a human life time. Published in *Vaccine (2003) S235-S237.* In 2002, together with Nadia Bernasconi, she received the ASIRB – Roche Research Award for the work on «Polyclonal activation of human memory B cells and maintenance of serological memory».

«Chemokines beyond agonistic activities – receptor antagonism and chemokine synergism»

Vibor Petkovic

University of Bern, Switzerland – 2004

Dr. Vibor Petkovic graduated in biology at the University of Zagreb, Croatia. In 2001 he entered the PhD program of the IRB in the group of Dr. Basil Gerber. He successfully defended his thesis at the University of Bern under the co-supervision of Prof. Clemens Dahinden, from the same university. During his PhD training he has investigated the interplay of chemokines in the regulation leukocyte trafficking during inflammation. The abundance of chemokines and chemokine receptors supports the importance of the chemokine system as a whole. Even though the effect of different chemokines one by one is very well understood, much less is known about the potential consequences of multiple chemokines expression in physiology and pathology. The detailed study of structural features of chemokines showed similarities among selective agonists and known natural antagonists, thus indicating this analysis as an additional instrument for disclosing the potential of different chemokines as natural antagonists. Combining these studies with *in vitro* analysis of chemokine activities on several chemokine receptors, Vibor described two novel natural chemokine antagonists: I-TAC and eotaxin-3. Noteworthy, eotaxin-3 is the first human chemokine that features broadband antagonistic activities suggesting that, in contrast to the other members of this family, it may have a role as amodulator chemokine rather than mediating inflammatory responses. In addition, he contributed to the discovery of a novel regulatory mechanism, employing synergy-inducing chemokines to amplify of leukocyte responses.
His results are published in *J. Biol. Chem. (2004) 279: 23357-23363; J. Leuk. Biol. (2004) 76:701-708; Blood (2005)105:3405-3412.*

Samantha Paoletti

«Fine tuning modulation of chemokine activities»

University of Fribourg, Switzerland – 2004

Dr. Samantha Paoletti graduated in biology at the University of Bologna. In 2001 she entered the PhD program of the IRB in the group of Dr. Mariagrazia Uguccioni. In May she successfully defended her thesis at the University of Fribourg under the co- supervision of Prof. Sandro Rusconi from the same university. The migration of leukocytes in immune surveillance and inflammation is largely determined by their response to chemokines. While the chemokine specificities and expression patterns of chemokine receptors are well defined, it is still a matter of debate how leukocytes integrate the messages provided by different chemokines that are concomitantly produced in physiological or pathological situations *in vivo*. She has been extensively investigated chemokine expression in human autoimmune diseases as well as in B cell tuomours developed at extranodal sites. The studies are published in *Arthritis Rheum. (2002) 46:3201-3211; Arthritis Rheum. (2004) 50:112-122; Eur. J. Immunol. (2005) 35:1347-1359; Blood (2003) 101:815-821.* She has also described a novel natural chemokine antagonist: eotaxin-3. This study is published in *Blood (2003) 102:789-794, J. Biol. Chem. (2004) 279: 23357-23363.*

Noteworthy, during her PhD training she has described a novel regulatory mechanism of leukocyte trafficking, based on chemokine-induced synergism. This mechanism provides an amplification system in inflammatory conditions where a «chemokine-rich» tissue can render leukocytes more competent to respond to migratory cues. These studies are published in *Blood (2005) 105:3405-3412.*

Simona Porcellini

«Role of calreticulin in T cell activation»

University of Milan, Italy – 2004

Dr. Simona Porcellini graduated in biology at the University of Milan. She entered the PhD program in Biotechnology at the University of Milan. In November 2004 she defended her thesis on the role played by calreticulin (CRT) in regulating T cell activation. In October 2002 she joined the group of Dr. Fabio Grassi at the IRB where she studied CD4 T cell response in recombinase-deficient fetal liver chimeric mice generated with CRT-deficient hemopoietic progenitors. Since CRT is the major Ca^{2+} binding protein in the endoplasmic reticulum (ER) and calcium signalling is involved at multiple stages of T cell development she hypothesized that CRT deficiency could have an impact on Ca^{2+} dependent T cell responses. She described the abnormal activation of CRT-deficient T cell in the periphery and their potential role in determining the immunopathology observed in CRT-deficient chimeras, thereby suggesting a mechanism of T cell responsiveness modulation through Ca^{2+} buffering in the ER.

«Glycoprotein folding, quality control and degradation: studies in chaperone-depleted cells and in cells with detective regulation of the unfolded protein response»

Klara Kristin Eriksson

ETH of Zurich, Switzerland – 2004

Dr. Klara Kristin Eriksson graduated in molecular biology at the University of Stockholm and entered the PhD program of the IRB in 2001 in the group of Dr. Maurizio Molinari. In September 2001 she successfully defended her thesis with a public presentation entitled *Glycoprotein folding, quality control and degradation: studies in chaperone-depleted cells and in cells with defective regulation of the unfolded protein response* at the ETH in Zurich. Prof. Ari Helenius (ETH Zurich), Prof. Roberto Sitia (DiBiT, Milan) and Prof. Ulrike Kutary (ETH Zurich) were external referents of Klara's thesis. She investigated molecular aspects of protein folding in the living cells and in particular the intervention in the process of several cellular factors such as molecular chaperones and folding enzymes. Her work was published in *Mol. Cell (2004) 13:125-135, J. Biol. Chem (2004) 279:44600-44605.*

«Complementarity, specialization and synergy among Toll-like receptors in human dendritic cells»

Giorgio Napolitani

University of Siena, Italy – 2004

Dr. Giorgio Napolitani graduated in Biology at the University of Padua. He started in April 2000 his doctorate at the Department of Evolutionary Biology at the University of Siena working at the Immunology Department at Chiron Vaccines Research Center in Siena, under the supervision of Prof. Cosima T. Baldari. In November 2002 he joined the group of Prof. Antonio Lanzavecchia at IRB and in February 2004 he successfully defended his thesis. At the beginning of his PhD Giorgio Napolitani worked together with David Jarrossay on the specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells [published in *Eur. J. Immunol. (2001) 31:3388-3393*]. Then he investigated the mechanisms involved in microbial recognition by dendritic cells. He defined a role for *src* kinases in the induction of cytokine production by LPS [published in *Eur. J. Immunol. (2003) 33:2832-2841*]. Finally, he tested the hypothesis that distinct TLRs able to recognize different microbial compounds can cooperate in dendritic cell activation. He found that specific combinations of TLR agonists potently synergize in the induction of the T_H1 polarizing factors IL-12, IL-23 and Delta-4. Using a microarray analysis approach he showed that TLR synergy had a selective effect in gene expression since it boosted only approximately 1% of the transcripts induced by single TLR agonists. These results identify a combinatorial code by which dendritic cells discriminate pathogens and provides a new concept for the rationale design of adjuvants. The results of this work are now in press in *Nature Immunology (2005).*

Laurie Chicha

«Immune cell development from early human hematopoietic progenitors»

University of Fribourg, Switzerland – 2005

Dr. Laurie Chicha graduated in biology at the university of Nice. In 2001 she entered the PhD program of the IRB in the group of Dr. Markus Manz. In June 2005 she successfully defended her thesis under the co-supervision of Prof. Sandro Rusconi at the University of Fribourg. Laurie studied the development of human natural interferon producing cells (IPCs, or plasmacytoid dendritic cells) and dendritic cells (DCs) form hematopoietic stem and progenitor cells first in vitro, and then in vivo. She established a highly innovative in vitro model to generate human IPCs and DCs, as well as B cells at the same time from$CD34^+$ hematopoietic stem and progenitor-cells *in vitro*. She then used this system to test the long-standing notion that IPCs and DCs are derived from either lymphoid or myeloid progenitor cells. She showed that both progenitor types are capable to produce both offspring IPCs and DCs on a clonal level. Laurie furthermore showed that human cord blood hematopoietic cells transplanted in the liver of newborn immunodeficient mice can reconstitute a human adaptive immune system with the development of IPCs and DCs. Her work was published in *J Exp Med (2004) 200: 1519-24* and *Science (2004) 304: 104-7.*

David Jarossay

«Functional study of human circulating dendritic cells: specialization and complementarity»

University of Fribourg, Switzerland – 2005

Dr. David Jarrossay entered the PhD program of the IRB in the group of Prof. Antonio Lanzavecchia. In June 2005 he successfully defended his thesis under the co-supervision of Prof. Sandro Rusconi at the University of Fribourg. David studied the expression of Toll-like receptors (TLR1 through TLR9) and the regulation of chemokine receptors and cytokine production in response to different microbial stimuli in two subsets of human peripheral blood DC. He found that myeloid DC express all TLR with the exception of TLR7 and TLR9, which are the only TLRs expressed by plasmacytoid DC. Furthermore myeloid and plasmacytoid DC respond to microbial products according to their TLR expression. In response to the appropriate stimuli both DC types up-regulate CCR7, a receptor that drives DC migration to the T cell areas. Type I IFN was produced only by plasmacytoid DC and at early time points after stimulation. Furthermore, its production was elicited by some of the maturation stimuli tested. These results published in *Eur. J. Immunol. (2001) 31:3388-3393*, have revealed a remarkable specialization and complementarity in microbial molecule recognition as well as a flexibility in effector function among myeloid and plasmacytoid DC.

Seminars and Courses at the Institute

2000

Alberto Mantovani, Mario Negri Institute, Milan, Italy
«Decoy receptors as a strategy to tune cytokines and chemokines» (20.07.00)

Roberto Gherzi, Department of Pharmacology, University of California, San Diego, CA, USA
«Proteins involved in activation-induced IL 2 mRNA stabilization» (21.07.00)

Costantino Pitzalis, Rheumatology Unit, Guy's Hospital, London, UK
«Mechanisms of lymphocyte migration in chronic arthropathies» (03.08.00)

Georges J. M. Maestroni, Cantonal Institute of Pathology, Locarno, Switzerland
«Neuroimmunomodulation and dendritic cell function» (07.08.00)

Myriam Capone, Ludwig Istitute for Cancer Research, Lausanne, Switzerland
«Analysis of NK T Cell Development in mice transgenic for human TCR Va24 chain» (13.10.00)

Ruggero De Maria, Laboratory of Hematology and Oncology, Istituto Superiore di Sanità, Rome, Italy
«Role of proapoptotic and antiapoptotic proteins in autoimmunity and tissue homeostasis» (18.10.00)

Sandro G. Rusconi, Institute of Biochemistry, Sciences Faculty, University of Fribourg, Switzerland
«Reconciling gene-regulation and gene-transfer studies» (20.10.00)

Jörg Kirberg, Basel Institute for Immunology, Switzerland
«T cell receptor specific clonal competition limits peripheral homoestatic proliferation in a hierarchical order» (24.10.00)

Jonathan Sprent, Department of Immunology, The Scripps Research Institute, La Jolla, CA, USA
«Cytokines and T cell memory» (10.11.00)

Gary Brewer, Department of Molecular Genetics and Microbiology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA
«Regulation of mRNA decay by the RNA-binding protein AUF.1» (10.11.00)

Martin Bachmann, Cytos Corporation Zurich, Switzerland
«T cell activation by CTLA-4: a paradigm revisited» (13.11.00)

Lorenzo M. Leoni, Department of Medicine, University of California, San Diego, CA, USA
«Mechanisms of lymphocyte depletion after treatment of B-chronic lymphocytic leukemia with Etodolac, a nonsteriodal anti-inflammatory agent» (16.11.00)

Vincenzo Barnaba, La Sapienza University of the Studies of Rome, Department of Internal Medicine, Umberto I Polyclinic, Italy
«Immunity, immunopathology, and autoimmunity: understanding their relation to viral infections» (20.11.00)

Bettina Ernst, Institute for Functional Genomics (GNF), San Diego, CA, USA
«Role of self peptide-MHC complex in homeostatic T cell proliferation and maintenance of a diverse T cell repertoire» (28.11.00)

Guido Poli, AIDS Immunopathogenesis Unit, DIBIT – San Raffaele Scientific Institute, Milan, Italy
«TCR-independent replication of CCR5-using HIV. A key to HIV pathogenesis?» (13.12.00)

Davide Robbiani, Cornell University, New York, USA
«Role of Multidrug Resistance Related Protein in the Lymphatic Mobilization of skin dendritic Cells» (18.12.00)

Sanjiv Luther, University of California, San Francisco, CA, USA «Chemokines and their roles in lymphocyte migration and activation» (19.12.00)

Matteo Bellone, Laboratory of Tumor Immunology, Cancer Immunotherapy and Gene Therapy Program, San Raffaele Scientific Institute, Milan, Italy
«Apoptosis, cross-priming, and the shaping of antigen-specific immune responses» (21.12.00)

2001

Rita Carsetti, Tor Vergata University of the Studies of Rome, Italy
«Lack of a defined population of B cells connecting the innate and adaptive immunity in the absence of the spleen» (17.01.01)

Augusto Gallino and Agostino Faggiotto, Cardiology, San Giovanni Hospital, Bellinzona, Switzerland
«Arteriosclerosis» (19.01.01)

Marco A. Cassatella, Institute of General Pathology, University of Verona, Italy
«Molecular bases regulating the responsiveness of neutrophils to IL-10» (05.02.01)

Anne Krug, Central Medical Clinic, University of Munich, Germany
«A new CpG oligonucleotide sequence induces maximal production of type I interferon in plasmacytoid dendritic cells» (01.03.01)

Deborah Braun, Gulbenkian de Ciência Institute, Oeiras, Portugal «Type I interferons, B cell activation and autoimmunity» (02.03.01)

Daniel C. Hössli, Pathology, Faculty of Medicine, University of Geneva, Switzerland
«Signaling through rafts / microdomains: modulation by transmembrane proteins» (09.03.01)

Pico Caroni, Friedrich Miescher Institute, Basel, Switzerland
«Plasticity and actin cytoskeleton regulation in the nervous system» (28.03.01)

Benedikt Kessler, Harvard University, Department of Pathology, Harvard Medical School, Boston, MA, USA
«New molercular probes which interfere with the ubiquitin-proteasome pathway and MHC class I antigen presentation» (29.03.01)

Brian Hemmings, Friedrich Miescher Institute, Basel, Switzerland
«Some new insights into the regulation of the PKB/AKT signalling pathway» (03.04.01)

Cosima T. Baldari, Department of Evolutionary Biology, University of Siena, Italy
«Interplay between lipid rafts and the actin cytoskeleton in TCR signalling» (11.04.01)

Christoph. B. Schmidt-Weber, Swiss Institute for Allergy and Asthma Research, Davos, Switzerland
«In human T cells the tumor suppressor PTEN is under control of costimulation and regulates proliferation by antagonizing PI3K» (18.04.01)

Annalisa Macagno, St. Gallen Cantonal Hospital, Switzerland
«Proteasomes in dendritic cells: alterations induced by maturation stimuli» (03.05.01)

Anna Morelli, University of Ferrara, Italy
«Purinergic receptors in immune cells: what are they and what do they do?» (25.05.01)

Hans-Uwe Simon, Pharmacological Institute, University of Bern, Switzerland
«Caspase-independent signaling pathways and cellular functiions initiated by death receptor activation» (28.05.01)

Facundo D. Batista, Medical Research Council, Laboratory of Molecular Biology, Cambridge, UK
«B cell response to antigen» (12.06.01)

Ed Palmer, Basel Institute for Immunology, Switzerland
«Dissecting survival and apoptotic signals from the T cell receptor» (15.06.01)

Oreste Acuto, Institut Pasteur, Paris, France
«T lymphocyte costimulation» (25.06.01)

Dario Neri, Department of Applied Biosciences, Swiss Federal Institute of Technology, Zurich, Switzerland
«Antibody-based targeting of angiogenesis» (04.07.01)

Markus Manz, Weissman Laboratory, Department of Pathology and Developmental Biology, Stanford University School of Medicine, Stanford, CA, USA
«Lineage committed hematopoietic progenitor cells in mouse and man» (06.07.01)

Kirsten Hammond, Department of Pathology and Immunology, Monash University Medical School, Melbourne, Australia
«NKT cells in normal and autoimmune disease-prone mice» (16.07.01)

Anna Mondino, DIBIT – San Raffaele Scientific Institute, Milan, Italy
«Characterization of tumor-specific CD4+ T cell responses during the establishment of solid tumors» (17.07.01)

Elissa Deenick, Centenary Institute of Cancer Medicine and Cell Biology, Sydney, Australia
«The role of IL-2 in the survival and proliferation of T lymphocytes – a modelling approach» (30.07.01)

Claudia Giachino, Maugeri Foundation, Pavia, Italy
«CTL responses to melanocyte differentiation antigens» (06.08.01)

Nicola Harris, Malaghan Institute of Medical Research, Wellington, New Zealand
«CD4+ T cell division, cytokine production and fate are determined by tissue microenvironment» (08.08.01)

Stuart Tangye, Centenary Institute of Cancer Medicine and Cell Biology, Sydney, Australia
«Intrinsic differences in human naïve and memory B cell proliferation and division-linked differentiation to plasma cells» (13.08.01)

Simon A. Jones, Cardiff School of Biosciences, Molecular Cell Biology Research Group, Cardiff University, UK
«Regulation of leukocyte recruitment by IL-6 and its soluble receptor» (23.08.01)

Charles R. Mackay, The Garvan Institute for Medical Research, Sydney, Australia
«B cells and the pathogenesis of autoimmune disease» (07.09.01)

Hermann Wagner, Institute for Medical Microbiology, Immunology and Hygiene, The Technical University of Munich, Germany
«Visualisation of CpG/TLR9/MYD88 signalosomes» (17.09.01)

Barbara Moepps, Department of Pharmacology and Toxicology, University of Ulm, Germany
«Chemokines and chemokine receptors of the lower vertebrate Xenopus laevis» (23.10.01)

Daniel F. Legler Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland
«Role of lipid rafts in CTL activation» (13.11.01)

Silvia Sebastiani, 'Istituto Dermopatico dell'Immacolata', Rome, Italy
«Effector and regulatory cells in allergic contact dermatitis» (21.11.01)

Paola Ricciardi-Castagnoli, Department of Biotechnology and Bioscience, Bicocca University of Milan, Italy
«A key role of IL-2 in early immune responses» (12.12.01)

Salvatore Oliviero, Deparment of Molecular Biology, University of Siena, Italy
«Function and regulation of VEGF-D and identification of new endothelial markers» (20.12.01)

2002

Matthias Clauss, Molecular Cell Biology, Max-Planck-Institute for Physiological & Clinical Research, Bad Nauheim, Germany
«New insights into endothelial cell activation» (08.01.02)

Luciano Adorini, Roche Milano Ricerche, Italy
«Induction of tolerogenic dendritic cells in the treatment of allograft rejection and autoimmune diseases» (14.01.02)

Giuliana Cassese, Deutsches Rheuma Forschungs Zentrum (DRFZ), Berlin, Germany
«Plasma cell survival niches in lymphoid and chronically inflamed tissues» (22.01.02)

Alberto Mantovani, Departement of Immunology and Cell Biology, Mario Negri Institute for Pharmacological Researches, Milan, Italy
«The role of the long Pentraxin in innate immunity» (23.01.02)

Walter G. Ferlin, Centre National de la Recherche Scientifique, Valbonne, France
«Investigating T cell tolerance in non obese diabetic (NOD) mice» (25.01.02)

Jeremy Luban, Departments of Microbiology and Medicine, Columbia University, New York, USA
«Cyclophilin A function in HIV-1 replication and in CD4+ T cells» (28.01.02)

Jacob Rachmilewitz, Goldyne Savad Institute for Gene Therapy, Hadassah University Hospital, Jerusalem
«Temporal summation of early signalling intermediates produced by successively triggered T-cell receptors» (29.01.02)

Mario Mondelli, Department of Infectious diseases, University of Pavia, Italy
«Humoral immune response to hepatitis C virus and viral variability» (05.02.02)

Roberto Gherzi, National Institute for Cancer Research, Genova, Italy
«Rapid degradation of unstable transcripts. The Exosome-AUBPs connection» (19.02.02)

Matthias Peter, Swiss Institute for Experimental Cancer Research (ISREC), Lausanne, Switzerland
«MAP kinase dynamics in yeast» (22.02.02)

Eli E. Sercarz, La Jolla Institute for Allergy and Immunology, San Diego, CA, USA
«Driver clones in autoimmunity and their regulation» (25.02.02)

Andrew Ziemiecki, Department of Clinical Studies, University of Bern, Switzerland
«Ephs and ephrins in mammary gland biology» (06.03.02)

Mathias Hornef, MTC/Karolinska Institute, Stockholm, Sweden
«LPS recognition in intestinal epithelial cells: balance between necessity and risk» (08.03.02)

James Sutton, University College London, UK
«B cell developments - the role of TGF-Beta RII» (11.03.02)

Ueli Aebi, Biozentrum, M. E. Mueller Institute for Structural Biology, University of Basel, Switzerland
«Task-sharing between actin and actin-binding proteins: who is doing what?» (20.03.02)

Roberto B. Cattaneo, Professor of Biochemistry and Molecular Biology, Mayo Medical School, Mayo, Minneapolis, USA
«Recombinant viruses for cytoreductive therapy» (28.03.02)

Christoph Moroni, Institute for Medical Microbiology, University of Basel, Switzerland
«Role of BRF1 in cytokine mRNA turnover» (02.04.02)

Brigitta Stockinger, Division of Molecular Immunology, The National Institute for Medical Research, London, UK
«Survival, homeostasis and competition in naïve and memory T cell pools» (22.04.02)

Tim H. Brümmendorf, Hematology/Oncology, University of Tuebingen, Germany
«Telomere length dynamics in normal hematopoiesis and in disease states associated with increased stem cell turnover» (29.04.02)

Wilhelm Krek, Growth Control Program, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
«SCF ubiquitin protein ligases: their roles in cell cycle control and cancer» (15.05.02)

Matthias Wymann, Institute of Biochemistry, Fribourg, Switzerland
«An integrator of chemokine and GPCR signalling: the lipid kinase PI3Kg» (24.05.02)

Massimo Levrero, La Sapienza University of Rome, Italy
«Modulation of the p53-related p73 function by post-translational modifications» (27.05.02)

Olav Zilian, Swiss Institute for Experimental Cancer Research (ISREC), Molecular Oncology, Epalinges s/Lausanne, Switzerland
«Requirements for Numb in directing precursor to mature cells during mouse development» (03.06.02)

Luca Maria Gambardella, Carlo Lepori, Dalle Molle Institute of Studies on Artificial Intellingence (IDSIA), Manno, Switzerland
«Swarm intelligence» (04.06.02)

Bernhard Moser, Theodor Kocher Institute, University of Bern, Switzerland
«Follicular homing T cells» (07.06.02)

Hidde L. Ploegh, Department of Pathology, Harvard Medical School, Boston, MA, USA
«Antigen presentation in real time» (10.06.02)

Ralf Küppers, Department of Internal Medicine, University of Cologne, Germany
«Aspects of B cell development and lymphoma genesis in the human» (11.06.02)

Riccardo Dalla-Favera, Institute for Cancer Genetics, Columbia University, New York, USA «Molecular pathogenesis of B cell lymphoma» (11.06.02)

Fabio Grassi, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA, USA «Self-limited life span of the pre-T cell receptor» (14.06.02)

Tobias Junt, Institute for Experimental Immunology, Department of Pathology, University of Zurich, Switzerland «Impact of CCR7 and its ligands on antiviral immune responses in vivo» (17.06.02)

Ulf Forssmann, IPF PharmaCeuticals GmbH, Hannover, Germany
«CCL14/HCC-1, a molecule gets active» (20.06.02)

Francesco Colotta, Molecular Oncology, Dompé Pharmaceuticals, L'Aquila, Italy
«Drug discovery applied to GPCRs: repertaxin as a novel inhibitor of IL-8 activity in post-ischemia reperfusion injury» (18.07.02)

Francesco Di Virgilio, University of Ferrara, Italy
«Extracellular nucleotides, P2 receptors and immune regulation» (19.07.02)

Gerd Sutter, Institute for Molecular Virology, Munich, Germany
«Replication-deficient vaccinia virus MVA as candidate vaccine against viral disease and cancer» (22.07.02)

Jutta Kollet, University of Nebraska Medical Center, USA
«IFN-gamma and LPS inducible recruitment of transcription factors to the proximal Interleukin-12 p35 promoter» (30.07.02)

Hongmin Li, Wadsworth Center, New York State Department of Health, Albany, NY, USA «The structural basis of T cell activation by superantigens» (14.08.02)

David N. Posnett, Cornell University, Weill Medical College, New York, USA «Significance of oligoclonal expansions of antigen specific CD8+ T cells» (19.08.02)

Walter E. Laug, Division of Hematology-Oncology, Children's Hospital Los Angeles, CA, USA
«Effect of av-integrin antagonists on orthotopic and heterotopic brain tumor growth» (19.08.02)

Jeffrey V. Ravetch, The Rockefeller University, New York, USA «Inhibitory signaling modulates peripheral tolerance» (21.08.02)

Giampietro Corradin, Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland
«Use of long synthetic poly-peptides for the rapid screening and development of malaria vaccine candidates» (23.08.02)

Ken Shortman, The Walter and Eliza Hall Institute Melbourne, Australia
«The influence of microbial stimuli on the development of murine dendritic cell subtypes » (02.09.02)

Claudio Basilico, Department of Microbiology, New York University School of Medicine, New York, USA
«Regulation of bone development by FGF signaling» (01.10.02)

Scott Mueller, Department of Microbiology and Immunity, The University of Melbourne, Australia
«Rapid CTL activation following cutaneous HSV-1 infection due to early antigen presentation in the draining lymph nodes» (02.10.02)

Philip M. Murphy, Laboratory of Host Defenses, NIAID, NIH, Bethesda, MD, USA
«Potential role for the fractalkine receptor CX3CR1 in human atherosclerosis» (10.10.02)

Michael Reth, University of Freiburg, Germany
«Early events in BCR signaling» (11.10.02)

Fabio Re, Dana-Farber Cancer Institute, Boston, MA, USA «Toll-like receptor in dendritic cell activation» (16.10.02)

Rafal Pacholczyk, Institute of Molecular and Genetics, Medical College of Augusta, Georgia, USA
«Visualization of the immunological synapse» (23.10.02)

Emanuela Corsini, Department of Pharmacological Sciences, University of Milan, Italy «Impairment in protein kinase C activation in aging» (12.11.02)

Lorenzo Leoni, Department of Medicine, University California San Diego, La Jolla, CA, USA «The role of cell-to-cell interaction and microenvironment in the pathogenesis of B cell malignancies» (18.11.02)

Roberto Sitia, DIBIT, San Raffaele Scientific Institute, Milan, Italy «Making and maintaining an efficient antibody factory» (11.12.02)

Salvatore Valitutti, INSERM, Institut Claude de Préval, Toulouse Cedex, France «On the functional role of the T cell/APC immunological synapse» (19.12.02)

2003

Marco Baggiolini, University of Lugano «Chemokines: 15 years after IL-8» (22.01.03)

Elodie Belnoue, Department of Immunology, Institut Cochin, Paris, France
«Leukocyte migration to the brain in experimental cerebral malaria» (24.01.03)

Ronald N. Germain, Laboratory of Immunology, NIAID, NIH, Bethesda, MD, USA
«T cell – dendritic cell interactions: dynamic visualization in lymphoid tissue and on the role of self-recognition» (07.02.03)

Steve Pascolo, Department of Immunology, Institute for Cell Biology, University of Tuebingen, Germany
«Stabilized mRNA as a vaccine vehicle and an adjuvant» (21.02.03)

Matthias Edinger, Department of Hematology and Oncology, University of Regensburg, Germany
«CD4+CD25+ regulatory T cells in murine models of allogeneic BMT: differential effect on graft-versus-host disease and graft-versus-tumor effect» (24.02.03)

Immanuel F. Luescher, Ludwig Institute for Cancer Research, Epalinges, Switzerland
«Role of CD8 and beta integrins in CTL activation» (12.03.03)

Simon Rothenfusser, Department of Clinical Pharmacology, University of Munich, Germany
«CpG-A and CpG-B: functional characterisation of two distinct types of immunostimulatory CpG oligonucleotides» (13.03.03)

Hans Wigzell, Microbiology and Tumor Biology Center, Karolinska Institutet, Stockholm, Sweden
«Immune protection or enhancement of infection against Chlamydia pneumoniae» (27.03.03)

Isabelle Maridonneau-Parini, Institute for Pharmacology and Structural Biology CNRS, Toulouse, France
«Role of tyrosine kinase Hck-positive lysosomes in the formation of podosomes» (01.04.03)

Ari Helenius, Institute of Biochemistry, ETH Hoenggerberg, Zurich, Switzerland
«What viruses teach us about endocytosis» (02.04.03)

Martin Bachmann, Cytos Biotechnology AG, Zurich, Switzerland
«From cross-presentation to cross-priming» (14.04.03)

Michael O. Hottiger, Institute of Veterinary Biochemistry and Molecular Biology, University of Zurich, Switzerland
«Role of the poly(ADP-ribose)polymerase-1 in NF-kB dependent gene expression» (29.04.03)

Marco E. Bianchi, DIBIT, San Raffaele Scientific Institute, Milano, Italy
«Passive release of chromatin protein HMGB1 from necrotic cells, and active secretion of HMGB1 by myeloid cells, triggers inflammation and primes dendritic cells for immune activation» (06.05.03)

Werner Reutter, Institute for Molecular Biology and Biochemistry, FU Berlin, Germany
«Biological implications of N-acyl neuraminic acid modifications and their role in T cell activation» (08.05.03)

Peter Gierschik, Department of Pharmacology and Toxicology, University of Ulm, Germany
«Regulation of Phospholipase C-beta isozymes by heterotrimeric and Rho GTPases» (12.05.03)

Lee-Ann Allen, Department of Internal Medicine, University of Iowa, USA
«Perturbation of phagocyte function by Helicobacter pylori» (15.05.03)

Ernesto Carafoli, Department of Biological Chemistry, University of Padua, Italy
«The control of cellular $Ca2^{+}$ signalling: Focus on membrane transporters» (22.05.03)

Andrew J. Pollard, Department of Paediatrics, University of Oxford, UK «Glyconjugate vaccines – how much do we know?» (06.06.03)

Silvano Sozzani, University of Brescia, Italy
«Role of PI3Kg in dendritic cell biology» (23.06.03)

Thomas Hartung, European Centre for the Validation of Alternative Methods ECVAM, CCR, Ispra, Italy
«Endotoxic properties of lipoteichoic acids» (01.07.03)

Simona Ferrari, Laboratory of Medical Genetics, University of Bologna, Italy
«Molecular anatomy of the CD40 and AID genes: the Hyper-IgM syndrome» (08.07.03)

Giampaolo Merlini, Department of Biochemistry, University of Pavia, Italy
«Systemic amyloidosis: diagnosis and therapy» (09.07.03)

Raffaele Badolato, Paediatric Clinic, University of Brescia, Italy
«Defects of innate immunity in primary immunodeficiencies» (10.07.03)

Anne O'Garra, National Institute for Medical Research, London, UK
«Development and function of IL-10 producing regulatory T cells: Comparison with other T Regs» (18.07.03)

Roberto B. Cattaneo, Mayo Clinic Rochester, MN, USA
«Measles virus biology: how to make a therapeutic agent from a pathogen» (14.08.03)

Dan R. Littman, Department of Pathology and Microbiology, New York University School of Medicine, New York, NY, USA
«Why do NKT cells patrol liver sinusoids?» (04.09.03)

Beat Imhof, Department of Pathology, University of Geneva, Switzerland
«The migration process of leukocytes» (05.09.03)

Nagata Kazuhiro, Department of Molecular and Cellular Biology, Institute for Frontier Medical Sciences, Kyoto University, Japan
«EDEM as one of key molecules in ER-associated degradation» (05.09.03)

Giuseppina Bonizzi, Department of Pharmacology, School of Medicine, UCSD, La Jolla, CA, USA
«IKK and the control of innate and adaptive immunity» (25.09.03)

Antonius Rolink, Department of Immunology, University of Basel, Switzerland
«Molecular mechanisms guiding early lymphocyte development» (26.09.03)

Manolis Pasparakis, EMBL Mouse Biology Program, Monterotondo (Rome), Italy
«In vivo analysis of NF-kB function by conditional targeting of IKK subunits» (03.10.03)

Alexandra Flemming, Lymphocyte Interaction Laboratory, Cancer Research, London UK
«SLP-65: an adapter protein functions as a tumor suppressor in pre-B cells» (10.10.03)

Marco Colonna, Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO, USA
«Interferon producing cells turn on NK cell recognition of virus» (03.11.03)

Harald von Boehmer, Department of Pathology, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA, USA
«Origin and lifestyle of regulatory T cells» (11.11.03)

Jagadeesh Bayry, INSERM U430, Institut des Cordeliers, Paris, France
«Natural antibodies and dendritic cells: maintenance of immune homeostasis» (26.11.03)

Marco Gattorno, »G. Gaslini» Scientific Institute, Genoa, Italy
«Synovial enrichment of interferon-a producing cells in juvenile idiopathic arthritis» (16.12.03)

2004

Giselle Sáurez Martinez, Center of Molecular Immunology, Havana, Cuba
«Ganglioside GM3 based cancer vaccine: a targeted therapy in cancer treatment» (08.01.04)

Charles R. Mackay, The Garvan Institute, for Medical Research Sidney, NSW, Australia «New ideas for the pathogenesis of asthma and autoimmune disease» (23.01.04)

Margot Thome-Miazza, Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland «Regulation of T-cell activation by Carma1 and Bcl10» (30.01.04)

Afonso Almeida, Lymphocyte Population Biology Unit, Institut Pasteur, Paris, France «CD4 T cell homeostasis: the thymus, the cells and the environment» (20.02.04)

Roger M. Nitsch, Division of Psychiatry Research, University of Zurich, Zurich, Switzerland «Vaccination against beta-amyloid in Alzheimer disease» (24.02.04)

Cesare Montecucco, Department of Experimental Biomedical Sciences, University of Padua, Italy «Immunosuppressive and proinflammatory activities of the VacA toxin of Helicobacter pylori» (10.03.04)

Roberto F. Speck, Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland «Immune-based therapies for controlling HIV: in vitro studies» (12.03.04)

Linda Erkman, Department of Medicine, UCSD, La Jolla, CA, USA «Role of a molecular pathway regulated by the POU domain transcription factor Brn-3.2/Brn-3b/Pou4f2 in axon guidance» (15.03.04)

Stephen P. Schoenberger, Division of Cellular Immunology, La Jolla Institute for Allergy and Immunology, San Diego, CA, USA «First impressions count: programming of CD8 T cell responses» (22.03.04)

Ivan Monteleone, Department of Internal Medicine, Tor Vergata University, Rome, Italy «Th1 differentiation in celiac disease intestinal mucosa» (06.04.04)

Cécile Bouneaud, Department of Immunology, Institut Pasteur, Paris, France «Central and effector memory CD8 T cells: lineage relationship, homeostasis and recall capacities in vivo» (22.04.04)

Gianvito Martino, Department of Neuroscience, DIBIT, San Raffaele Hospital, Milan, Italy «The neuroprotective role of neural stem cells in multiple sclerosis» (22.04.04)

William M. Nauseef, Inflammation Program and Department of Medicine, Roy J. and Lucille A. Carver College of Medicine, University of IOWA, USA «Myeloperoxidase: aspects of synthesis, structure and function» (23.04.04)

Alan Tyndall, University of Basel, Basel, Switzerland «Hematopoietic stem cell transplantation for severe autoimmune disease: results and future directions» (07.05.04)

Marek Cebecauer, Ludwig Institute for Cancer Research, Epalinges, Switzerland «Soluble dimeric peptide/MHC-I molecules: good or bad guys for the effector CD8+ T cells?» (14.05.04)

Jürg Tschopp, Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland «The inflammasome: a complex sensing bacteria and causing IL-1β activation» (18.05.04)

Elisabetta Dejana, IFOM-FIRC Institute of Molecular Oncology, Milan, Italy «Endothelial cell-cell junctions: happy together» (27.05.04)

Bruno Sobral, Virginia Bioinformatics Institute at Virginia Tech, Blacksburg, VA, USA «PathoSystems – an integrated experimental, computational, modeling and theoretical approach to infectious diseases» (17.06.04)

Sylvie Bertholet, NIH/NIAID/LPD, Bethesda, MD, USA «Pathogens, phagosomes, and antigen cross-presentation: a new pathway» (25.06.04)

Jeremy Luban, Departments of Microbiology and Medicine, Columbia University, College of Physicians and Surgeons, New York, NY, USA «Cyclophilin and innate resistance to HIV-1» (08.07.04)

Cindy Ma, Centenary Institute of Cancer Medicine and Cell Biology, Newtown, NSW, Australia «Impaired humoral immunity in XLP in vivo is due to defective T-cell help» (10.08.04)

Guido Poli, San Raffaele Scientific Institute, DIBIT; Milan, Italy «New insights on HIV-T cell interaction» (02.09.04)

Edecio Cunha-Neto, Division of Clinical Immunology, Heart Institute, University of São Paulo, Brazil «Do inflammatory cytokines alter cardio myocyte gene and protein expression in Chagas disease?» (03.09.04)

Caterina Strambio de Castillia, Laboratory of Cellular and Structural Biology, The Rockefeller University, New York, NY, USA «The peripheral nuclear MIp network promotes the structural integrity and function of the spindle pole body, the yeast microtubule organizing center» (06.09.04)

Vincenzo Bronte, Department of Oncology and Surgical Sciences, University of Padua, Italy «L-arginine metabolism in CD11b+/Gr-1+ myeloid cells restrains T lymphocyte responsiveness to the antigen» (15.09.04)

Michael Karin, University of California San Diego, USA. 2004-2005 PhD Lecture course. «The IKB kinase complex at the crossroads of inflammation and cancer» (01.10.04)

Steven L. Reiner, Department of Medicine, Division of Infectious Diseases, University of Pennsylvania, Philadelphia, PA, USA «Fashioning and re-fashioning cell fate in the immune response» (11.10.04)

Lloyd Ruddock, Biochemistry Department, Linnanmaa Campus, University of Oulu, Finland «Small steps towards understanding the human-PDI family» (13.10.04)

Clemens Dahinden, Institute for Immunology, University of Bern, Switzerland «Inflammatory and immunoregulatory cells in Th2-type immune responses» (15.10.04)

Didier Trono, Department of Microbiology and Molecular Medicine, University of Geneva, Switzerland. 2004-2005 PhD Lecture course. «Defensive arts: innate intracellular defenses against retroelements» (26.10.04)

Hans Acha-Orbea, Department of Biochemistry, University of Lausanne, Switzerland «Histiocytosis and B lymphocyte hyperproliferation: two new models to probe the immune response» (10.11.04)

Dirk Busch, Institute for Medical Microbiology, Immunology and Hygiene, Technical University Munich, Germany. 2004-2005 PhD Lecture course. «Generation of protective immunity against intracellular bacteria» (11.11.04)

Giuseppe Vassalli, Faculty of Biology and Medicine, University of Lausanne, Switzerland «Gene therapy approaches to cardiovascular disease» (12.11.04)

Cosima T. Baldari, Department of Evolutionary Biology, University of Siena, Italy «Modification of T-cell antigen receptor signalling by bacterial toxins» (15.11.04)

John Telford, Chiron Vaccines, Siena, Italy «Virulence factors and vaccine candidates of group B Streptococcus» (15.11.04)

Francesco Blasi, Università Vita Salute San Raffaele, Milan, Italy «Il recettore dell'urochinasi (uPAR): ruolo nella migrazione e nella proliferazione cellulare» (16.11.04)

Vincent Piguet, University Hospital, Geneva, Switzerland «HIV-1 escape from immune responses: A role for dendritic cells and viral gene products» (26.11.04)

Alicia Maria Hidalgo Estevez, Centro de Biologia Molecular »Severo Ochoa», Madrid, Spain «HIV-1 tat increases NFAT-AP-1 cooperation» (09.12.04)

K. George Chandy, Department of Physiology and Biophysics, University of California Irvine, USA «Potassium Channels as Targets for Specific Immunomodulation of memory cells: new therapy for autoimmune disease» (10.12.04)

2005

Luca G. Guidotti, Immunopathogenesis of Liver Infections Unit, DIBIT, San Raffaele Scientific Institute, Milan, Italy «A crucial role for platelets in modulating T cell-mediated antiviral immunity and immunopathology» (11.01.05)

Kurt Ballmer-Hofer, Paul Scherrer Institut, Molecular Cell Biology, Villigen-PSI, Switzerland. 2004-2005 PhD Lecture course. «From Receptor/Ligand Interaction to Modulation of Cell Junctions» (18.01.05)

Philippe Moingeon, Research and Development Stallergenes, Antony, France «Challenges and opportunities in the development of recombinant allergy vaccines» (24.01.05)

Marco Bianchi, Molecular Biology, San Raffaele University, Milan, Italy «HMGB1 is the signal for traumatic cell death» (31.01.05)

Klaus Uberla, Department of Molecular and Medical Virology, Ruhr-University Bochum, Germany. 2004-2005 PhD Lecture course. «Lentiviral vectors for gene therapy and vaccination» (08.03.05)

Albrecht Wendel, University of Konstanz, Biochemical Pharmacology, Konstanz, Germany «Membrane-bound TNF mediates melphalan hepatotoxicity via activation of both TNF receptors» (11.03.05)

Carlomaurizio Montecucco, Cattedra e Struttura Complessa di Reumatologia, Scuola di Specializzazione in Reumatologia, Università di Pavia, Policlinico S. Matteo, Pavia, Italy «Rheumatoid arthritis: clinical perspectives» (16.03.05)

Gerardo Z. Lederkremer, Department of Cell Research and Immunology, Faculty of Life Sciences, Tel Aviv University, Israel
«Distinct sugar chain processing and sorting in ER quality control» (04.04.05)

Werner Muller-Esterl, Institute for Biochemistry, University of Frankfurt Medical School, Frankfurt, Germany.
2004-2005 PhD Lecture course.
«The nuts and bolts of the nitric oxide (NO) signaling cascade» (07.04.05)

Florian Bihl, Partners AIDS Research Center, Massachussetts General Hospital, Harvard Medical School, Charlestown, Boston, USA
«Immune dominant cytotoxic T lymphocyte responses in viral co-infections» (07.04.05)

Bernhard Moser, Institute of Cell Biology, University of Bern, Switzerland
«Role of chemokines in antigen presentation» (12.04.05)

Grazia Galli, Immunology and Virology Unit, Chiron Vaccines Srl, Siena, Italy
«Acquiring from the innate: NKT cells help to B cell responses» (13.04.05)

Fulvio Reggiori, University of Michigan, Life Sciences Institute, Ann Arbor, USA
«Membrane trafficking during autophagosome formation» (15.04.05)

Nick Huntington, Immunology Division, Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia
«Regulation of B and NK cell immune responses by CD45» (25.04.05)

Rhys Allan, Department of Microbiology and Immunology, University of Melbourne, Australia
«Re-examining the Role of Skin Dendritic Cells in CTL Immunity to HSV-1 infection» (27.04.05)

Antal Rot, Novartis Institute for Biomedical Research, Vienna, Austria.
2004-2005 PhD Lecture course.
«Chemokine Interceptors: Silent molecules with different functions» (11.05.05)

Tanja Hartmann, Department of Internal Medicine, University Hospital Freiburg, Germany
«CXCR4 chemokine receptor and b1 signaling cooperate in mediateing adhesion and chemoresistance in small cell lung cancer and chronic lymphocytic leukaemia» (13.05.05)

Sanjiv Luther, Department of Biochemistry, University of Lausanne, Epalinges, Switzerland
«Lymphoid tissue development and cell migration» (23.05.05)

Antonio G. Siccardi, DIBIT-HSR, Milan, Italy
«IgE enhance the immunogenicity of cellular tumor vaccines» (01.06.05)

Jens V. Stein, Theodor Kocher Institute, University of Bern, Switzerland
«Intracellular control of lymphocyte trafficking» (02.06.05)

Lars Hviid, Centre for Medical Parasitology, Department of Infectious Diseases, Rigshospitalet, Copenhagen, Denmark «Malaria Immunology» (14.06.05)

Michael T. Lotze, Translational Research, Molecular Medicine Institute, University of Pittsburgh School of Medicine, Pittsburgh, USA
«Dying Dangerously: Apoptosis, Necrosis and Cancer» (15.06.05)

Symposia, Meetings, Seminars and Courses

2000

The NIH Wednesday Afternoon Lecture, Bethesda, USA
A. Lanzavecchia: «From Synapses to Immunological Memory»

Midwinter Conference of Immunologists, Asilomar, CA, USA
A. Lanzavecchia: «Control of T lymphocyte activation and migration»

Lecture at the Department of Cell Biology, University of Yale, USA
A. Lanzavecchia: «Memory T cells»

EMBO course «Cell migration in inflammation and immunity», HSR-Milan, Italy
A. Lanzavecchia: «T cell activation and migration»

CNRS Institute of Molecular and Cell Biology, Strasbourg, France
A. Lanzavecchia: «Dendritic cells»

Transfusion Medicine 2000, Cambridge, UK
F. Sallusto: «Dendritic cells in primary, effector and memory immune reponses»

Swiss Society for Allergology and Immunology, Basle, Switzerland
A. Lanzavecchia: «T lymphocyte activation, traffic and effector functions»

Arthur Levin Memorial Lecture, Guy's, King's and St. Thomas' School of Medicine, London, UK
A. Lanzavecchia: «Dendritic cells: from infection to cancer»

6th National Symposium Basic Aspects of Vaccines, Walter Reed Army Institute of Research, Bethesda, USA
A. Lanzavecchia: «On the cellular basis of immunological memory»

5th Congress of The European Society of Contact Dermatitis, Amsterdam, The Netherlands
F. Sallusto: «Chemokines and dendritic cell maturation»

German Society for Immunology, Workshop «Cytokines in inflammation», Lubeck, Germany
F. Sallusto: «A chemocentric view of the immune response: insight into the mechanism of T cell priming and the nature of immunological memory»

Workshop on HIV/AIDS Vaccine Development, National Agency for AIDS Research, Paris, France
A. Lanzavecchia: «Dendritic cells and antigen presentation»

ENII conference, «Molecular regulation of lymphocyte behaviour», Ile des Embiez, France
F. Sallusto: «Regulation of T cell priming and polarization by dendritic cells»

6th International Symposium on Dendritic Cells, Port Douglas, Australia
A. Lanzavecchia: «DC migration and maturation»
F. Sallusto: «Control of T cell immunity by DC»

7th Annual Meeting «Innate Immunology in Transplantation», Nantes, France
A. Lanzavecchia: «Chemokines in innate immunity»

SI/SIIIC/BCG/EAACI joint meeting, Ferrara, Italy
A. Lanzavecchia: «T cell priming by dendritic cells»

Congress on «AIDS, cancer and hepatitis: scientific and ethical challenges», San Marino, RSM
A. Lanzavecchia: «The role of dendritic cells in antigen presentation and T cell priming»

International Congress of Cell Signalling in the Immune System, Genoa, Italy
A. Lanzavecchia: «From Synapses to Immunological Memory»

7th Workshop on Human Leucocyte Differentiation Antigens, Harrogate, UK
M. Uguccioni: «Cytokine/chemokine receptors»

Annual European Congress of Rheumatology, Nice, France
A. Lanzavecchia:»The role of chemokines receptor in the orchestration of the immune response»

Course on «Basic Immunology», University of Bologna, Italy
M. Uguccioni: «Biological activities of chemokines»

Department of Internal Medicine, Bologna, Italy
M. Uguccioni: «Chemokine expression in inflammatory disease»

International Society of Cancer Gene Therapy, Paris, France
A. Lanzavecchia: «The dynamics of T lymphocyte activation»

XIXth Congress of the European Academy of Allergy and Clinical Immunology, Lisbon, Portugal
F. Sallusto: «Dendritic cells»
F. Sallusto: «Chemokines receptors»

Institute for Microbiology and Hygene, University of Marburg, Germany
F. Sallusto: «Chemokine receptors in primary, effector and memory immune responses»

18th International Congress of Biochemistry and Molecular Biology, Birmingham, UK
A. Lanzavecchia: «Regulation of chemokine receptor expression in T lymphocytes and dendritic cells»

Gordon Conference 'Chemotactic Cytokines', Kimball Union Academy, USA
A. Lanzavecchia: «Regulation of chemokine receptor expression in T lymphocytes and dendritic cells»

Annual Meeting British Society for Allergy and Clinical Immunology, Birmingham, UK
F. Sallusto: «Dendritic cells in primary, effector and memory immune responses»

XVIII International Congress of the Transplantation Society, Rome, Italy
A. Lanzavecchia: «Regulation of T lymphocyte priming and polarization by dendritic cells»

Joint Swiss-Japanese Scientific Seminar, Ermatingen, Switzerland
A. Lanzavecchia: «From the immune synapse to T cell fate determination»

2000 ELSO Meeting, Geneva, Switzerland
A. Lanzavecchia: «Immunological memory harbored by distinct T cell subsets»

Immunology Student Retreat Meeting, Harvard Medical School, Boston, MA, USA
A. Lanzavecchia: «T lymphocyte activation and differentiation»

European Virology 2000, Glasgow, UK
M. Molinari: «Folding and quality control of viral glycoproteins in the endoplasmic reticulum»

FEBS International Summer School on Immunology, Ionian Village, Greece
A. Lanzavecchia: «TCR triggering and T cell activation» and «Memory T cells»

Baltic Summer School, «Current trends in immunology and signal transolution», Kiel, Germany
Federica Sallusto: «The role of dendritic cells in the generation of effector and memory T cell responses»

1st Lemanic Meeting on Chemokines and Chemokine Receptors, Geneva, Switzerland
A. Lanzavecchia: «Regulation of chemokine receptor expression in T lymphocytes and dendritic cells»

14th European Immunology Meeting, Poznan, Poland
A. Lanzavecchia: «T-cell activation»

From the Laboratory to the Clinic, Trinity College, Oxford, UK
F. Sallusto: «Activated T cells and exhausted dendritic cells»

XXXVII Congress of the European Renal Association, Nice, France
M. Uguccioni: «Chemokine receptors blockers»

Heremans Lecture, University of Brussels, Belgium
A. Lanzavecchia: «From the immune synapse to T cell fate determination»

XI AINI meeting, Camogli, Italy
A. Lanzavecchia: «Dynamics of T lymphocyte responses: intermediates, effector and memory Cells»

2nd Colloque, Centre Lemanique de Recherche sur le SIDA, Lausanne, Switzerland
A. Lanzavecchia : «Regulation of chemokine receptor expression in T lymphocytes and dendritic cells»

Grabar Lecture, French Society of Immunology, Paris, France
A. Lanzavecchia: «The dynamics of T lymphocyte activation»

Immunological correlates of protection from HIV infection and disease, Sestri Levante, Italy
F. Sallusto: «Role of dendritic cells in primary, effector and memory T cell responses»

Annual Immunology Research Day, Guy's, King's and St. Thomas' School of Medicine, London, UK
A. Lanzavecchia: «The role of dendritic cells in T cell activation and differentiation»

Annual Congress, British Society for Immunology, Harrogate, UK
A. Lanzavecchia: «The dynamics of T lymphocyte activation»

Gulbenkian de Ciência Institute, Oeiras, Portugal
M. Thelen: Lectures on signal transduction
G. Natoli: Lectures of transcriptional regulation

Cours d'Immunologie approfondie, Institut Pasteur, Paris, France
A. Lanzavecchia: Lecture on «Dendritic cells and T cell priming»

2001

DIBIT Congress Center San Raffaele, Milan, Italy
M. Molinari: «Calreticulin loss prevents virus entry and affects glycoprotein maturation»

Lecture at the Master in Biotechnologies, Bicocca University of Milan, Italy
A. Lanzavecchia: Lecture on «Dendritic cells»

Cancer and the Cell Cycle, Lausanne, Switzerland
M. Thelen: «Nuclear localization of HsPI3K-C2a»

EAACI Meeting - Basic Immunology in Allergy and Clinical Immunology, Davos, Switzerland
A. Lanzavecchia: «T cell activation and generation of memory/effectors cells»

Keystone Symposium, Chemokines and Chemokine Receptors, Taos, New Mexico, USA
A. Lanzavecchia: «Chemokine receptors in primary and secondary immune responses»

Gordon Research Conference, Immunochemistry & Immunobiology, Ventura, CA, USA
A. Lanzavecchia: «Generation and maintenance of effector and memory T cells»

Keystone Symposiun, Dendritic cells: interfaces with immunobiology and medicine, Taos, New Mexico, USA
A. Lanzavecchia:»The dynamics of T lymphocyte activation»
F. Sallusto: «The role of chemokines and chemokine receptors in primary, effector and memory immune responses»

3rd Symposium on Clinical Immunology of Southern Switzerland, Lugano, Switzerland
A. Lanzavecchia: «La presentazione dell'antigene al sistema immunitario»

Symposium of the International Federation for Clinical Chemistry, Mainz, Germany
J. Geginat: «Cytokine-driven proliferation and differentiation of human CD4+ T cells: a model for T cell homeostasis»

Course on Fundamental Virology, Institut Pasteur, Paris, France
M. Molinari: «The folding of viral glycoproteins in the endoplasmic reticulum»

Seminar at the Institut Pasteur, Paris, France
M. Molinari: «Calreticulin loss affects glycoprotein maturation in the endoplasmic reticulum»

Seminar at the University of Alberta, Edmonton, Canada
M. Molinari: «Calreticulin loss prevents virus entry and affects glycoprotein maturation»

American Association of Immunologists Annual Meeting, Experimental Biology 2001, Orlando, FL, USA
A. Lanzavecchia: «From T cell stimulation to generation of effector and memory cells»
F. Sallusto: «A chemocentric view of the immune response»

IPF PharmaCeuticals GMBH, Hannover, Germany
B. Gerber: «Molecular mechanisms of chemoattractant receptor recognition and activation»

Course on «Basic and clinical allergy», Imperial College, London, UK
F. Sallusto: «Chemokines»

Course on «Cellular and molecular infection biology», Karolinska Institute, Stockholm, Sweden
F. Sallusto: «Role of dendritic cells in the induction and regulation of cellular immunity»

ENII Conference 2001, Ile des Embiez, France
A. Lanzavecchia: «Migration of lymphocyte populations»

Annual Meeting of SII, Abano Terme, Italy
M. Thelen: «CXCR4 mediated signal transduction»

Merck Research Laboratories, West Point, NY, USA
A. Lanzavecchia: «Role of chemokine receptors in the immune response»

Nobel Symposium «Global HIV Therapeutics – HIV Vaccines», Stockholm, Sweden
A. Lanzavecchia: «Generation and maintenance of effector and memory T cells»

Symposium on Molecular Cell Biology of Macrophages 2001, University of Tokyo, Japan
A. Lanzavecchia: «T lymphocyte activation, differentiation and migration»

Ruggero Ceppellini Course: «Remembering environmemtal experiences», Capo Miseno, Naple, Italy
A. Lanzavecchia: «On the cellular basis of immunological memory»

Course on «Basic Immunology», University of Bologna, Italy
M. Uguccioni: «Biological activities of chemokines»

11th International Congress of Immunology, Stockholm, Sweden
A. Lanzavecchia: «Stimulation and expansion of dendritic cells»
F. Sallusto: «Origin and migration of dendritic cells»

RWTH, Aachen, Germany
M. Thelen: «SDF-1 induces prolonged signaling of CXCR4»

Transcriptional regulation in eukaryotes, Cold Spring Harbor, NY, USA
G. Natoli: «Recruitment of the NF-kB to chromatin targets: constitutive versus regulated accessibility»

Seminar at the Institute Giannina Gaslini, Genoa, Italy
A. Lanzavecchia: «Dendritic cells»

Seminar at the Centre d'Immunologie, Marseille Luminy, France
A. Lanzavecchia: «T lymphocytes-dendritic cell interactions: intermediates, effectors and memory cells»

Fifth World Congress on Inflammation, Edinburgh, UK
F. Sallusto: «Regulation of T cell immunity by dendritic cells»

Seminar at the University of Lausanne, Switzerland
M. Thelen: «CXCR4 mediated signal transduction»

Antibody Club Meeting, British Society for Immunology, London, UK
A. Lanzavecchia: Keynote address «Regulation of T cell immunity by dendritic cells»

Seminar at the Bernhard Nocht Insititute for Tropical Medicine, Hamburg, Germany
M. Uguccioni: «Natural chemokine antagonists»

Seminar at the Free University of Berlin, Institute For Microbiology, Berlin, Germany
M. Uguccioni: «Chemokine expression in human disease»

Euresco Conference «Comunicaton within the immune system: basic rules and their breakdown», San Felieu de Guixols, Spain
F. Sallusto: «Dendritic cells and the generation of effector and memory T cells»

Congress on Cell therapy «Filling the gap between basic science and clinical trials», Istituto Superiore di Sanità, Rome, Italy
A. Lanzavecchia: «Dendritic cells and immunotherapy»

«Vaccines of the future: from rational design to clinical development», Institut Pasteur EuroConferences, Paris, France
A. Lanzavecchia: Keynote address: Generation of effector and memory T cells»

International Symposium on Immunoallergology, Molecular Pathology and Clinic of Immunoallergology, Savigliano, Italy
A. Lanzavecchia: «Sottopopolazioni di linfociti T della memoria con funzioni effettrici distinte»

Falk Symposium Autoimmune Diseases in Pediatric Gatroenterology, Basle, Switzerland
A. Lanzavecchia: «From antigen presentation to protective immunity»

Immunology symposium, University of Bern, Switzerland
F. Sallusto: «T lymphocyte priming: from non-effector to effector and memory cells»

Gulbenkian de Ciência Institute, Oeiras, Portugal
M. Thelen: «Lectures on signal transduction»
G. Natoli: «Lectures on transcriptional regulation»

31st Annual Meeting of the Japanese Society for Immunology, Osaka, Japan
F. Sallusto: «Dendritic cells and the generation of effector and memory T cells»

Seminar at the University of Kyoto, Japan
F. Sallusto: «Pathogen discrimination by dendritic cells»

Francophone Club of dendritic cells, Paris, France
J. Geginat: «TCR-independent proliferation and differentiation of human CD4+ T cells: a role for dendritic cells?»

2002

Lymphocyte Traffic and Homeostasis, Newport Beach, CA, USA
A. Lanzavecchia: «Subsets of memory T cells»

Seminar at the Cerus Corporation, Concord, USA
A. Lanzavecchia: «From antigen presentation to protective immunity»

Keystone Symposium, Innate Immunity: Evolution and link to adaptive immunity, Taos, USA
A. Lanzavecchia: «Keeping up with T and B cell memories»

Seminar at the Memorial Sloan Kettering Cancer Center, New York, USA
A. Lanzavecchia: «Keeping up with T and B cell memories»

3rd Internatonal Congress on Autoimmunity, Geneva, Switzerland
M. Uguccioni: «Chemokines as target for inhibition»

Seminar at the University of Milan, Department of Pharmacological Sciences, Italy
A. Lanzavecchia: «On the cellular basis of immunological memory»

Cologne Spring Meeting 'Immunity', Cologne, Germany
A. Lanzavecchia: «How is memory maintained in the immune system?»

USGEB Congress 2002, Lugano, Switzerland
M. Molinari: «The molecular chaperones BiP and PDI mediate endoplasmic reticulum associated protein degradation»

Seminar at the University of Fribourg, Switzerland
M. Thelen: «Chemokine mediated signal transduction»

Seminar at the University of Gent, Belgium
G. Natoli: «The impact of chromatin dynamics on NF-kB dependent transcription»

Course on Fundamental Virology 2002, Institut Pasteur, Paris, France
M. Molinari: «The folding of viral glycoproteins in the endoplasmic reticulum»

The Henry G. Kunkel Lecture 2002, Johns Hopkins University, Baltimore, USA
A. Lanzavecchia: «How is memory maintained in the immune system»

Fifth International Calreticulin Workshop, S. Antonio, Texas, USA
M. Molinari: «Glycoprotein folding and quality control in the endoplasmic reticulum: consequences of calreticulin or calnexin deficiency»

Seminar at the University of Padova, Italy
M. Molinari: «Endoplasmic reticulum-associated protein degradation»

Joint Meeting of the IRB and Virginia Tech, Riva San Vitale, Ticino, Switzerland
A. Lanzavecchia:»Induction of protective memory by vaccination»
F. Sallusto: «T cell priming by dendritic cells»
M. Thelen: «Chemokine receptor-mediated signal transduction»
B. Gerber: «Structure-fucnction relationship in chemokines»
G. Natoli: «Chromatin dynamics in the inflammatory response»
K. Karjalainen: «BAC transgenesis»

Seminar at the Theodor Kocher Institute, University of Bern, Switzerland
G. Natoli: «The activation of inflammatory genes by NF-kB: the impact of chromatin dynamics»
P. Ogilvie: »Natural chemokine antagonists»

Seminar at the Institute for Rheumatology, University of Pavia, Italy
M. Uguccioni: «Chemokine expression in inflammation»

36th Annual Meeting of ESCI, Phagocyte Workshop, Brussels, Belgium
M. Thelen: «Chemokine receptor mediated signal transduction»

«Immune mechanism and disease», Grenada, West Indies
A. Lanzavecchia: «On the cellular basis of immunological memory»

«Immune memory», Lunteren, The Netherlands
A. Lanzavecchia: «Memory stem cells»

«Cytokines as natural adjuvants: perspectives for vaccine development», Istituto Superiore di Sanità, Rome, Italy
A. Lanzavecchia: «Interactions between dendritic cells and T cells»

Lectures at the University of Siena, Italy
A. Lanzavecchia: «B cells lymphomas»; «Transplantation»; «Innate and adoptive immunity»

«The role of dendritic cells in Physiology and Pathology», Mario Negri Institute for Pharmacological Researches, Milan, Italy
F. Sallusto: «The role of dendritic cell subsets in the immune response»

Karolinska Institute, Course on Cellular and molecular infection biology, Section B: Immunoregulation of microbial infections, Stockholm, Sweden
F. Sallusto: «Role of dendritic cells in the induction and regulation of cellular immunity»

Forbeck Focus on the future meeting 2002, Deidesheim, Germany
J. Geginat: «Cytokines and the maintenance of T cell memory»

»HIV and HCV infections: anti-viral protection, virus-mediated damage and therapy», University of Parma, Italy
A. Lanzavecchia: «From priming to effector function and memory»

EMBO-Serono Foundation, «Lymphocyte antigen receptor and coreceptor signaling», Siena, Italy
A. Lanzavecchia: «Memory stem cells»

1st International Conference Italian Society of Immunology SIICA 2002, Montecatini Terme, Italy
A. Lanzavecchia: «From primary response to long term momory»

6th International Symposium of the Immunotherapy of the Rhematic Diseases, Cyprus
A. Lanzavecchia: «Memory stem cells»

American Society for Microbiology ASM 102nd General Meeting, «Immunobiology and role in infection and immunity», Salt Lake City, Utah, USA
A. Lanzavecchia: «Regulation of T cell immunity by dendritic cells»

XIII International Congress of Histocompatibility and Immunogenetics, «Adoptive immunity and therapeutic application», Seattle, Washington, USA
A. Lanzavecchia: «T lymphocyte activation by antigen presenting cells»

The Marcus Wallenberg Symposium at Nobel Forum Karolinska Institute, «Contemporary topics in immunology», Stockholm, Sweden
A. Lanzavecchia: «T cell recognition and activation»

XIV Pezcoller Symposium, «The novel dichotomy of immune interactions with tumors», Trento, Italy
A. Lanzavecchia: «How memories are kept in the immune system»

ENII Conference 2002, «Early and late: innate versus adaptive immune response», Ile des Embiez, France
G. Natoli: «Mechanisms regulating NF-kB recruitment to endogenous chromatin targets»

Université Pierre & Marie Curie, Premières Journées des Cordeliers «The latest advances in immonotherapy», Paris, France
F. Sallusto: «Control of T cell immunity by dendritic cells»

Seminar at the University of Catania, General Pathology, Italy
M. Thelen: «Chemokine receptor signal transduction»

Euroconference, «Interactions between innate and adaptive immunity in mammalian defense against bacterial infections», Flesensee, Germany
A. Lanzavecchia: «Dendritic cell responses»

8th International Conference of Malignant Lymphoma, Lugano, Ticino, Switzerland
A. Lanzavecchia: «Memory stem cells»

«The Impact of the Post-genomics Era on Immunology, Virology and Oncology», San Marino Republic
A. Lanzavecchia: «How memories are kept in the immune system»

Scientific Basis of Rheumatology, Royal College of Physicians, London, UK
A. Lanzavecchia: «Memory stem cells»

2nd Annual Meeting of the Federation of Clinical Immunology Societies FOCIS, San Francisco, CA, USA
A. Lanzavecchia: «Memory stem cells»

Seminar at the Tumour Institute of Genova (IST), Italy
G. Natoli: «Regulation of NF-kB recruitment to chromatin»

Seminar at the Institute for General Zoology, University of Muenster, Germany
M. Thelen: «Chemokine receptor signal transduction»

European Academy of Allergy and Clinical Immunology, XXIst Congress, Naples, Italy
F. Sallusto: «The biology of dendritic cells»

Course on «Basic Immunology», University of Bologna, Italy
M. Uguccioni: «Biological activities of chemokine»

Merieux Foundation Symposium «Therapeutic vaccines against HIV and cancers», Veyrier-du-Lac, Switzerland
F. Sallusto: «Exploiting dendritic cells for T cell priming»

1st Dr. Schleussner Symposium Frontiers in Immune Pharmacology, «The therapeutic use of chemokines and chemokine antagonists», Frankfurt am Main, Germany
F. Sallusto: «Cell migration in primary, effector ad memory immune responses»

«Euroconference: Interactions between innate and adaptive immunity», Goehren-Lebbin, Germany
J. Geginat: «DC, cytokines and the generation and maintenance of T cell memory»

Brainstorming Meeting on Allergy and Inflammation Studies, Bad Wiessee, Munich, Germany
A. Lanzavecchia: «On the cellular basis of immunological memory»

International Society for Experimental Hematology, 31st Annual Meeting, Montreal, Canada
F. Sallusto: «Memory stem cells»

Gordon Research Conference 'Chemotactic cytokines', Mount Holyoke College, MA, USA
M. Thelen: «Chemokine receptor signal transduction»
F. Sallusto: «Chemokine receptors in primary and effector T cell responses»

Seminar at the University of Palermo, Department of Biopathology, Italy
F. Sallusto: «The role of dendritic cells in the induction and regulation of the immune response»

International Course in Immunology, University of Chile, Santiago, Chile
F. Sallusto: «Regulation of T cell immunity by dendritic cells»; «T cell activation: from intermediates to effector and memory T cells»

XIX International Congress of The Transplantation Society, Miami, Florida, USA
A. Lanzavecchia: «Lymphocyte Homing»

«Abnormal Proteins in Neurodegenerative Disease Meeting», University of Zurich, Switzerland
M. Molinari: «EDEM, a novel component of the mammalian ER quality control machinery»

EMBL meeting on transcriptional regulation in eukaryotes, Heidelberg, Germany
G. Natoli: «Dynamic changes in histone H3 Lys9 methylation at tightly regulated inflammatory genes»

International Scientific Symposium, University of Bern, Switzerland
F. Sallusto: «Dendritic cells»

2nd Swiss-Japanese Scientific Seminar, 'Role of cyrtokines in the norm and in the disease', Tokyo/Nikko, Japan
A. Lanzavecchia: «Chemokines and immunity»

FEBS Intenational Summer School on Immunology, «The immune system: genes, receptors and regulation», Ionian Village, West Coast of Peloponese, Greece
A. Lanzavecchia: «T cell priming and deletion»; «Maintenance of serological memory»

7th International Symposium on Dendritic Cells, Bamberg, Germany
A. Lanzavecchia: «T cell priming and deletion by dendritic cells»

University of Vienna Medical School, «Allergology: from the past to future developments», Austria
A. Lanzavecchia: «Memory stem cells»

German Society for Immunology, Marburg, Germany
G. Natoli: «Activation of inflammatory genes by NF-kB: the impact of chromatin dynamics»

5th EFIS Tatra Immunology Conference 'Molecular determinants of T cell immunity', Tatranské Zruby, Kosice, Czech Republic
F. Sallusto: «Cytokine memory in human T lymphocytes»

9th Congress of the Italian Association for Transplantation Immunogenetics and Biology, Pesaro, Italy
M. Uguccioni: «The chemokine network»

'Karolinska Research Lectures at Nobel Forum', Karolinska Institute, Stockholm, Sweden
A. Lanzavecchia: «How is memory maintained in the immune system?»

The International Cytokine Society, Torino, Italy
A. Lanzavecchia: «Cytokines and immunological memory»

Gert Riethmüller Symposium, Munich, Germany
A. Lanzavecchia: «Immunological memories»

European Macrophage and Dendritic Cell Society EMDS, Basle, Switzerland
A. Lanzavecchia: «The impact of dendritic cells on T cell response»

Guru at the 2002 Annual NIH Immunology Interest Group, Airlie, Virginia, USA
A. Lanzavecchia: «On the cellular basis of the immunological memory»

Seminar at the University of Milan, Department of Pharmacology, Chemotherapy and Medical Toxicology «E. Trabucchi», Milan, Italy
F. Grassi: «Constitutive activation and extinction of the pre-T cell receptor»

Seminar at the Nestlé Research Center, Lausanne, Switzerland
F. Sallusto: «Regulation of T cell immunity by dendritic cells»

Gulbenkian de Ciência Institute, Oeiras, Portugal
M. Thelen: «Lectures on signal transduction»

Euroconference: Novel strategies of mucosal immunisation through exploitation of mechanisms of innate immunity in pathogen-host interaction, Siena, Italy
A. Lanzavecchia: «Innate immunity informs memory T cells»
F. Sallusto: «Immunological memory and vaccination»

DYNAL lecture, Institute of Immunology, University of Kiel, Germany
A. Lanzavecchia: «Memory stem cells»

Seminar at the Research Institute of Molecular Pathology IMP / Intercell, Vienna, Austria
A. Lanzavecchia: «Short term and long term serological memory»

Annual Meeting of the Austrian Society of Allergology and Immunology, Innsbruck, Austria
A. Lanzavecchia: «Short term and long term memory in the immune system»

EMBO Sectoral Meeting on Immunology, Lisbon, Portugal
A. Lanzavecchia: «Short term and long term serological memory»

Advanced Course in Immunology 2002-2003, Institut Pasteur, Paris, France
F. Grassi: «Development and selection of ab lymphocytes»

Seminar at the University of Zurich, Switzerland
G. Natoli: «Mechanisms of specificity in NF-kB-dependent activation of inflammatory genes»

Rene Touraine Foundation, Scientific Day 2002, Paris, France
F. Sallusto: «The role of chemokine receptors in primary, effector and memory immune responses»

Seminar at the Cancer Research UK, London Research Institute, London, UK
F. Sallusto: «Subsets of human memory T lymphocytes»

Seminar at the Windeyer Institute of Medical Sciences, Royal Free & University College Medical School, London, UK
A. Lanzavecchia: «From antigen presentation to immunological memory»

BSI/BSACI Joint Congress, Harrogate, UK
A. Lanzavecchia for the 2002 Jack Pepys Lecture
«How is memory maintained in the immune system»

2nd Conference on Vaccine, Istituto Superiore di Sanità, Rome, Italy
A. Lanzavecchia: «Immunological memory»

Advanced Course in Immunology 2002-2003, Institut Pasteur, Paris Cedex, France
A. Lanzavecchia: «T lymphocyte-dendritic cell interaction: intermediates, effectors and memory cells»

Seminar at the VIMM, Padova, Italy
A. Lanzavecchia: «Immunological memory»

Seminar at the Technical University of Munich, Germany
G. Natoli: «Specificity and redundancy in the NF-kB family of transcription factors»

2003

Seminars at the University of Milan, Department of Pharmacological Sciences, Italy
A. Lanzavecchia: «T lymphocyte activation»
F. Grassi: «The role of calnexin in thymocyte development»
M. Uguccioni: «Chemokine expression and function»

Seminar on Immunology for the Southern Switzerland Society of Dermatology and Venereology, Stabio, Switzerland
A. Lanzavecchia: «Immunology: dendritic cells»

Basic Virology Course, Institut Pasteur, Paris, France
M. Molinari: «The folding of viral glycoproteins in the endoplasmic reticulum»

«The application of gene therapy to leukemia and lymphoma» Workshop, Miami Beach, FL, USA
A. Lanzavecchia: «Strategies for overcoming immune tolerance in malignancy»

Keystone Symposium on Basic aspects of tumor immunology, Keystone, CO, USA
A. Lanzavecchia: «Migration and function of T cells in vivo»

XXX Seminar on Evolution and Biology, Molecules and diseases, Rome, Italy
M. Molinari: «The protein factory»

Meeting on Abnormal proteins in neurodegenerative disease, University of Zurich, Zurich, Switzerland
M. Molinari: «Role of EDEM in ER-associated protein degradation»

European School of Oncology Course, «Biology and treatment of malignant lymphomas», Monte Verità, Ascona, Switzerland
A. Lanzavecchia: «Dendritic cells and malignant lymphomas»
M. Manz: «Development of dendritic cells from hematopoietic stem- and progenitor cells»
M. Uguccioni: «Chemokine expression and activities in tumors»

35th Annual Meeting USGEB 2003, Davos, Switzerland
A. Lanzavecchia: «Common themes in T and B cell memory»

Cellular Therapy 2003: 2nd International Symposium on the Clinical Use of Cellular Products, Regensburg, Germany
M. Manz: «Human hematopoietic cell reconstitution in mice»

Annual Meeting of the German Society of Virology, Berlin, Germany
A. Lanzavecchia: «Dendritic cells as key players in antiviral immunity»

Keystone Symposium on Conformational diseases of the secretory pathway, Taos, New Mexico, USA
M. Molinari: «EDEM regulates release of misfolded glycoproteins from the calnexin cycle during ER quality control»

Keystone Symposium on Dendritic cells: interfaces with immunobiology and medicine, Keystone, CO, USA
G. Natoli: «NF-kB-dependent transcriptional control in dendritic cells»

Seminar series ‘Colloquium in molecular medicine’, Aachen University, Aachen, Germany
G. Natoli: «Mechanism underlying specificity in NF-kB-regulated transcription»

6th Winter Conference in Immunology, ‘Chemokines in Immunity’, St. Sorlin, France
M. Uguccioni: «Natural chemokine antagonists»

Conference on Translational research in autoimmunity, Portofino, Italy
A. Lanzavecchia: «Vaccination and immunological memory»
J. Geginat: «T cell fitness determined by signal strength»

Conference on The future of vaccines – Cancer meets infectious diseases, Semmering, Austria
A. Lanzavecchia: «Maintenance of serological memory»

Seminar at the University of Washington, Seattle, WA, USA
A. Lanzavecchia: «Vaccination and immunological memory»

Seminar at Institut Pasteur, Paris, France
F. Sallusto: «Regulation of dendritic cell and T cell migration in the immune response»

Sonderforschungsbereich des FWF: SFB F018 «Molecular and Immunological Strategies for Prevention, Diagnosis and Treatment of Type I Allergies», Vienna, Austria
F. Sallusto: «Subsets of human memory T lymphocytes»

Conference on Cell therapy: the state of the art and new perspectives, Pavia, Italy
A. Lanzavecchia: «On cellular basis of immunological memory»

Sixth Annual Conference on Vaccine Research, Arlington, VA, USA
A. Lanzavecchia: «Effector and memory T cells»

ENII Conference 2003, Molecular and cellular profiles of immune responses, Ile des Embiez, France
A. Lanzavecchia: «Impact of dendritic cell migration on T cell priming and immune responses»
F. Sallusto: «Human memory T lymphocyte subsets»

II Annual Congress of the Italian Society of Immunology on Clinical Immunology and Allergology, Verona, Italy
F. Sallusto: «From dendritic cell migration to T cell memory»

Lund Strategic Research Center for Stem Cell Biology and Cell Therapy, Lund University, Sweden
M. Manz: «Developmental pathways in early hematopoiesis»

Nobel Forum, Immunologic activation: rational design of vaccines and immunotherapeutics – An infection and vaccinology meeting, Karolinska Institute, Stockholm, Sweden
A. Lanzavecchia: «Activation, differentiation and memory of T and B cells»

15th European Immunology Congress, EFIS 2003, Rhodes, Greece
A. Lanzavecchia, Plenary Lecture: «Common themes in T and B cell memory»

Conference on Dendritic cells and oncology vaccination, Valencia, Spain
A. Martín-Fontecha: «Dendritic cell recruitment into lymphatics: regulation and impact on lymph node shut down and T cell priming»

European hematology association meeting, Lyon, France
M. Manz: «Dendritic cell development»

Seminar at Altana Pharma, Konstanz, Germany
M. Uguccioni: «Chemokines and chemokine receptors as targets in the treatment of human inflammatory disease»

XXII Congress of the European Academy of Allergy and Clinical Immunology, Paris, France
F. Sallusto: «Activation and polarization of T cells and dendritic cells»

Forth Expert Meeting on Clinical Dendritic Cell Immunotherapy, Amsterdam, The Netherlands
F. Sallusto: «Cascades of DC and T-lymphocyte trafficking regulated by cognate interactions and chemokines»

Seminar at the University of Palermo, Italy
F. Sallusto: «Migration of dendritic cells and T lymphocytes in the immune response»

Joint meeting UniPathology Zurich, IRB and IOSI, Monte Verità, Ascona, Switzerland
S. Didichenko: «The role of PI3-kinases in cell cycle regulation»
M. Molinari: «Protein folding and quality control in the endoplasmic reticulum»
M. Thelen: «Chemokine receptor mediated cell activation»
E. Traggiai: «Serological memory»

The Awaji International Forum on Infection and Immunity, Hyogo, Japan
J. Geginat: «Generation and maintenance of human memory T cell subsets»

28th Development Seminar, Novartis, Basle, Switzerland
M. Molinari: «BACE (beta-site amyloid precursor protein cleaving enzyme) inhibition as a potential disease modifying therapy of Alzheimer's disease»

International Scientific Symposium on Chronic inflammatory responses of the lung, Bern, Switzerland
M. Uguccioni: «Expression and function of chemokines in inflammation»

IMP special lecture in memoriam Laura Stingl, University of Vienna Medical School, Austria
A. Lanzavecchia: «Vaccination and immunological memory»

AIDS Vaccine 2003 Conference, New York, USA
A. Lanzavecchia: «On the cellular basis of serological memory»

11th Congress of the European Society for Organ Transplantation ESOT, Venice, Italy
A. Lanzavecchia: «Immune modulation by dendritic cells»

«Biopolo meets Lombardia», Swiss Center, Milan, Italy
A. Lanzavecchia: «The Institute for Research in Biomedicine»

Euresco Conference on Biology of molecular chaperones, Tomar, Portugal
M. Molinari: «EDEM regulates release of misfolded glycoproteins from the calnexin cycle during ER quality control»

Seminar at the School of Biomedical Sciences, Medical School, University of Nottingham, Nottingham, UK
M. Thelen: «Chemokine receptor signal transduction»

Signalling Program, Braham Institute, Cambridge, UK
M. Thelen: «HsPI3K-C2·: hints on its function»

Seminar at the Sir William Dunn School of Pathology, University of Oxford, Oxford, UK
M. Thelen: «Chemokine receptor signal transduction»

Foundation for the Medical Applied Research, University of Navarra, Pamplona, Spain
A. Martín-Fontecha: «Regulation of the immune system by dendritic cells: main characters and supporting actors»

Seminar at Glaxo Smith Kline, Stevenage, UK
F. Sallusto: «Regulated dendritic cell and T lymphocyte traffic in the immune response»

Seminar at the King's College London, Guy's Hospital, London, UK
F. Sallusto: «Subsets of human memory T lymphocytes»

34th Annual Meeting of the German Society of Immunology, Berlin, Germany
F. Sallusto, EFIS Lecture: «Cascades of DC and T-lymphocyte trafficking regulated by cognate interactions and chemokines»

'Cancer Vaccines 2003 – Cancer & HIV Vaccines: shared lessons', New York, USA
A. Lanzavecchia: «Vaccination and immunological memory»

'Dendritic cells: biology and therapeutic applications', Centre for International Meetings on Biology, Juan March Institute, Madrid, Spain
A. Lanzavecchia: «Regulation of T cell immunity by dendritic cells»

5th FISV Congress, Rimini, Italy
A. Lanzavecchia: «Vaccination and immunological memory»

II International Congress on Immunology and Clinical Immunology, «Immunology 2003: present evidences, future directions», Savigliano, Italy
A. Lanzavecchia: «On the cellular basis of immunological memory»
F. Sallusto: «Regulation and migration of dendritic cells and T lymphocytes in the immune response»

'Face to face SARS and Influenza', University of Milan, Italy
A. Lanzavecchia: «The role of antibodies and memory B cells in antiviral immunity»

Conference at the Medical Faculty of the University of Geneva, Switzerland
A. Lanzavecchia: «Vaccination and immunological memory»

Juan March Workshop on Dendritic cells: biology and therapeutic applications, Madrid, Spain
G. Natoli: «Transcriptional regulation in dendritic cells»

XXVIII Meeting of the Brazilian Society of Immunology, Mangaratiba, Brazil
F. Sallusto: «Antigen decoding by T lymphocytes»

34th International Symposium of the Princess Takamatsu Cancer Research Fund on Cancer immunotherapy, Tokyo, Japan
A. Lanzavecchia: «Vaccination and immunological memory»

Conference at SES (Società Elettrica Sopracenerina), Locarno, Switzerland
A. Lanzavecchia: «Biotechnologies and innovative vaccines: recent successes and new challenges»

Conference at Cardiocentroticino, Civico Hospital, Lugano, Switzerland
A. Lanzavecchia: «Translational research: new projects at the IRB»

Conference on Memory in history and nature, University of Siena, Italy
A. Lanzavecchia: «Memory in the immune system»

Conference at PLR (Partito liberale radicale ticinese), Bellinzona, Switzerland
A. Lanzavecchia: «Biomedical and biotechnological research in the Ticino Canton»

Advanced Course in Immunology, Institut Pasteur, Paris, France
F. Grassi: «Development and selection of αβ lymphocytes»

Seminar at the Utrecht University, Utrecht, The Netherlands
M. Molinari: «Protein degradation and protein secretion from the endoplasmic reticulum»

Seminar at the Swiss Institute for Pedagogy, Bellinzona, Switzerland
M. Molinari: «Biomedical research in Switzerland»

Seminar at the Instituto Gulbenkian de Ciencia, Oeiras, Portugal
M. Thelen: «Chemokine receptor signal transduction»

First Conference of the Swedish Infection Biology Network, Stockholm, Sweden
F. Sallusto: «Vaccination and immunological memory»

Advanced Course in Immunology, Institut Pasteur, Paris, France
A. Lanzavecchia: «T lymphocytes-dendritic cell interactions: intermediates, effectors and memory cells»

Department of Pathology, Stanford University, Stanford USA, December 12, 2003.
M. Manz: «Human adaptive immune system reconstitution in mice»

The 33rd Annual Meeting of the Japanese Society for Immunology, Fukuoka, Japan
A. Lanzavecchia: «Maintenance of serological memory»

International workshop on Gene expression control in haematolymphoid cells, University of Wuerzburg, Germany
G. Natoli: «Activation of inflammatory genes by NF-kB recruitment to chromatin targets»

Seminar at the Institute for Immunology, University of Bern, Switzerland
M. Thelen: «Chemokine receptor signal transduction»

XI Workshop on Advances in molecular biology for young researchers abroad, Madrid, Spain
A. Martín-Fontecha: «Influence of dendritic cells and NK cells in lymph node traffic»

Annual Meeting of the Dutch Society for Immunology, Noordwijkerhout, The Netherlands
F. Sallusto: «Regulation of dendritic cells and T cell migration in the immune response»

2004

Basic Virology Course, Institut Pasteur, Paris, France
M. Molinari: «The folding of viral glycoproteins in the endoplasmic reticulum»

Keystone Symposium on NF-kappaB: Biology and Pahtology, Snowbird, Utah, USA
G. Natoli: «The impact of chromatin organization on the NF-kB response»

Mikrobiologie Kolloquium 2004, Zurich, Switzerland
M. Molinari: «ER-associated protein degradation»

Seminar at the University of Basle, Advanced Immunology I Course, Basle, Switzerland
F. Sallusto: «Chemokines and chemokine receptors»

Seminars at the University of Milan, Department of Pharmacological Sciences, Italy
A. Lanzavecchia: «The research at the IRB»
F. Grassi: «T cell development»
M. Uguccioni: «Chemokine expression and function»

Seminar at IOSI, Bellinzona, Switzerland
M. Manz: «Human adaptive immune system reconstitution in mice»

Seminar at Boehringer Ingelheim, Biberach, Germany
M. Thelen: «HsPI3K-C2a: hints on its function»

Seminar at the Harvard Medical School, Boston, MA, USA
A. Lanzavecchia: «On the cellular basis of immunological memory»

Seminar at the Massachussetts General Hospital, Charlestown, MA, USA
A. Lanzavecchia: «On the cellular basis of immunological memory»

Seminar at the Istituto Giannina Gaslini, Genoa, Italy
M. Uguccioni: «Chemokine expression in inflammatory diseases and in tumours»

Gordon Research Conference on «Immunochemistry & Immunobiology», Buellton, CA, USA
A. Lanzavecchia: «B and T cell memory»

Seminar at the Paul Scherrer Institute, Villingen, Switzerland
M. Thelen: «Chemokine Receptor Signal Transduction»

63rd Annual Assembly of the Swiss Society for Microbiology, Lugano, Switzerland
A. Lanzavecchia: «Micro-organisms and the immune system»

6th Residential Course of Neuroimmunology, Bergamo, Italy
A. Lanzavecchia: «Mechanisms of autoantibody production»

Seminar at the Mount Sinai Hospital School of Medicine, New York, USA
M. Manz: «Dendritic cell development»

Seminar at the Blood Center, Havard School of Medicine, Boston, USA
M. Manz: «Immunoreconstitution of mice and men»

Swiss HIV Research Meeting, Crans Montana, Switzerland
M. Manz: «Human immune reconstitution in mice-new options for HIV research»

International Titisee Conference «From allergy to cancer: new perspectives for therapeutic vaccination», Titisee, Germany
A. Lanzavecchia: «Vaccination and immunological memory»

1st Swiss Workshop on Basic HIV Research, Crans Montana, Switzerland
A. Lanzavecchia: «Induction and maintenance of serological memory»

Immunology and Diabetes Society Conference 2004, Cambridge, UK
F. Sallusto: «The dendritic cell as a determinant of T cell phenotype»

Meeting with Science 2004, Bellinzona, Switzerland
M. Molinari: «Biomedical research at the IRB»

NCCR Neural Plasticity and Repair Symposium, Konstanz, Germany
M. Molinari: «Abnormal proteins and neurodegenerative diseases»

Keystone Symposium on «HIV vaccine development: progress and prospects», Whistler, British Columbia, Canada, USA
A. Lanzavecchia: «Induction and maintenance of serological memory»

ECVAM Workshop «Dendritic cells as a tool for a predictive identification of skin sensitization hazard», European Commission Joint Research Centre, Ispra, Italy
F. Sallusto: «Monocyte-derived dendritic cells: a model to study the effects of innate immunity on the acquired immune responses»

Annual Meeting Swiss Society of Allergology and Immunology, Geneva, Switzerland
F. Sallusto: «T cell activation and maintenance of memory»

Seminar at the Emory University, Atlanta, GA, USA
A. Lanzavecchia: «Maintenance of serological memory»

10th National Symposium on «Basic aspects of vaccines», Bethesda, MD, USA
A. Lanzavecchia: «Maintenance of serological memory»

Seminar at the Vaccine Research Center, Bethesda, MD, USA
A. Lanzavecchia: «Mechanisms that sustain serum antibody levels»

Sixth International Calreticulin Workshop, «Functions and Dynamics of ER/SR Proteins», Zermatt, Switzerland
M. Molinari: «EDEM regulates release of misfolded glycoproteins from the calnexin cycle during ER quality control»

20th Natural Killer Cell Workshop, Leeuwenhorst, The Netherland
A. Martín-Fontecha: «NK cells are recruited into lymph nodes by migrating dendritic cells and regulate CD4 T cell priming»

6th Calreticulin Workshop, Zermatt, Switzerland
F. Grassi: «Altered lymphocyte homeostasis by calreticulin deficiency»

Seminar at Novartis Forschungsinstitut, Wien, Austria
M. Thelen: «Chemokine Receptor Signal Transduction»

Medienkonferenz «Tage der Genforschung», Schweizer Gentage, Bern, Switzerland
M. Manz: «Das menschliche Immunsystem in der Maus: Schaffen wir neue ethische Probleme?»

Genedays 2004 «The biomedical research in Ticino, success and perspectives», Bellinzona, Switzerland
A. Lanzavecchia: «The Institute for Research in Biomedicine»

«Varese Pediatrics», Insubria University, Varese, Italy
A. Lanzavecchia: «The seroprophylaxis today»

Seminar at the Institut Pasteur, Paris, France
M. Manz: «Immunoreconstitution of mice and men»

Seminar at the Istituto G. Gaslini, Genoa, Italy
F. Sallusto: «Control of T cell immunity by dendritic cells»

First Meeting on «Protein Glycosylation and Disease», Bucharest, Romania
M. Molinari: «Mechanisms of glycoprotein degradation»

TuBS Symposium on Cell Trafficking in Health and Disease, Turku, Finland
F. Sallusto: «Dendritic cell traffic»

The 21st Sigrid Jusélius International Symposium on «Cell Trafficking in Inflammation and Cancer: a Round Trip between Tissues and Vessels», Helsinki, Finland
F. Sallusto: «Dendritic cell traffic»

Seminar at the Ulm University, Germany
M. Thelen: «Regulatory Circuits in Chemokine Receptor-mediated Signal Transduction»

Annual European Congress of Rheumatology, Berlin, Germany
F. Sallusto: «T lymphocytes as central regulators of immunity and immunopathology».

Seminar at Novartis Institutes for Biomedical Research, Basle, Switzerland
A. Lanzavecchia: «Exploiting immunological memory for vaccination and serotherapy»

XXIII EAACI Congress, Amsterdam, Holland
F. Sallusto: «T cell commitment: fixed or flexible?» and «Chemokines».

Meeting of the delegates of Labmed Switzerland, Ticino session, «The immune system», Monte Verità, Ascona, Switzerland
A. Lanzavecchia: «The lymphocytes: immunological monitoring»
M. Uguccioni: «The cellular migration: in vitro test and tissular evaluation»

Meeting on «Respiratory allergy: from basic immunology to bedside», Genoa, Italy
A. Lanzavecchia: «Vaccination and immunological memory»

EU Network Meeting on Cells as Protein Factories, Contea, Italy
M. Molinari: «EDEM couples protein degradation to protein folding and quality control»

11. Jahrestagung der Deutschen Gesellschaft für Gentherapie und 2. Workshop «Virale Vektoren» der Gesellschaft für Virologie (GFV), Georg-Speyer-Haus, Frankfurt, Germany
M. Manz: «Immunoreconstitution of mice and men»

First Novartis Lunch, Bellinzona, Switzerland
M. Molinari: «An alternative approach to reduce the generation of amyloid beta in vivo»

Seminar at the Centre d'Immunologie de Marseille-Luminy, CNRS-INSERM Université de la Mediterranée, Campus de Luminy, Marseille, France
M. Manz: «Immunoreconstitution of mice and men»

12th International Congress of Immunology and 4th Annual Conference of the Federation of Clinical Immunology Societies FOCIS, Montréal, Québec, Canada, USA
A. Lanzavecchia: «Lymphocyte activation for tolerance, effector function and memory»

Seminar at the Department of Immunology, University of Munich, Germany
M. Manz: «Immunoreconstitution of mice and men»

Montreal Meeting on Peptide Receptos, Montreal, Canada, USA
M. Thelen: «Regulatory Circuits in Chemokine Receptor-Mediated Signal Transduction»

Expert Panel in B Cell Biology Meeting, Park Ridge, NJ, USA
A. Lanzavecchia: «Exploring and exploiting human B cell memory»

Seminar at Wyeth Research, Pearl River, NY, USA
A. Lanzavecchia: «Exploring and exploiting human B cell memory»

AIDS Vaccine 2004 International Conference, Lausanne, Switzerland
A. Lanzavecchia: «Understanding and exploiting immunological memory»

FEBS International Summer School on Immunology, Western Peloponese, Greece
A. Lanzavecchia: «Leukocyte traffic in immune responses»

Advanced Course in Immunology, Institut Pasteur, Paris, France
A. Lanzavecchia: «T lymphocyte-dendritic cell interactions: intermediates, effector and memory cells»

Basic Course in Virology, Institut Pasteur, Paris, France
M. Molinari: «The folding of viral glycoproteins in the endoplasmic reticulum»

Helenius Symposium on the Endoplasmic Reticulum, Zurich, Switzerland
M. Molinari: «Beta-site specific intrabodies to decrease and prevent generation of Alzheimer's Abeta peptide»

VIII National Congress of the Italian Society for Experimental Hematology SIES, Pavia, Italy
A. Lanzavecchia: «Immunological memory»

Bioforum, 'Biotechnologies: where science meets business', Milan, Italy
A. Lanzavecchia: «Serotherapy and human monoclonal antibodies»

Hematopoietic Stem Cells V, Tübingen, Germany
M. Manz: «huAIS-RG-mice, a new model of the human hematolymphoid system»

Workshop 'Dendritic cells in cancer immunotherapy: from bench to bedside', IFOM, Milan, Italy
F. Sallusto: «Regulation of dendritic cell and T cell migration to draining lymph nodes»

SIICA Course «Cellule dendritiche regolatorie nel controllo della risposta immunitaria», Certosa di Pontignano, Italy
F. Sallusto: «Cellule dendritiche: eterogeneità di fenotipo e funzione»

Symposium on 'Emerging infections and new vaccinations', Marburg, Germany
A. Lanzavecchia: «Immunological memory and vaccination»

Seminar at the Max Planck Institute for Infection Biology Berlin, Germany
J. Geginat: «The role of cytokines in the generation and maintenance of human memory T cells»

Seminar at the Italian Society for Rheumatology, Stresa, Italy
M. Uguccioni: «Chemokines in inflammatory diseases»

First Basle Immunology Focus (BIF) Symposium, «Development of the lymphohematopoietic system», University of Basle, Switzerland
M. Manz: «Immunoreconstitution of mice and men»

Seminar at the Medizinische Hochschule Hannover, Germany
M. Thelen: «New Chemokine receptor-mediated signal transduction»

7th International Congress of Neuroimmunology, Venice, Italy
A. Lanzavecchia: «On the cellular basis of immunological memory»

Euroconferences, «Vaccines 3: Frontiers in vaccine development», Institut Pasteur, Paris, France
A. Lanzavecchia: «Vaccination and immunological memory»

8th International Symposium on Dendritic Cells, Brugge, Belgium
A. Lanzavecchia: «Dissection of pathways for dendritic cell activation»
F. Sallusto: «Control of dendritic cell migration and its impact on lymphocyte trafficking and T cell priming»

4th Benjamin Franklin Stem Cell Workshop, Charite University, Berlin, Germany
M. Manz: «Dendritic cell development from hematopoietic stem and progenitor cells – New options for targeted therapies?»

Annual Meeting of the Danish Society for Flow Cytometry, Aarhus, Denmark
J. Geginat: «Phenotypic characterization of central memory and effector memory T cells»

Seminar at the Department of Immunology, University of Zurich, Switzerland
M. Manz: «Reconstitution of the human adaptive immune system in mice»

Seminar at the Microbiology and Tumor Biology Center, Karolinska Institute, Stockholm, Sweden
M. Manz: «Immunoreconstitution of mice and men»

Seminar at the Accademia Medica di Roma, Policlinico Umberto I, Rome, Italy
A. Lanzavecchia: «The cellular basis of immunological memory».

Seminar at the Institute of Experimental Immunology, University Hospital, Zurich, Switzerland
A. Lanzavecchia: «Exploring and exploiting human B cell memory»

DNA Vaccines 2004 Conference, Monte Carlo, Monaco
A. Lanzavecchia: «Vaccination and immunological memory»

Seminar at the Institute for Hygiene, University of Innsbruck, Austria
M. Uguccioni: «Activities and expression of chemokines in secondary lymphoid organs upon vaccination for SIV»

Seminar at the Mediterranean Institute for Haematology, Rome, Italy
M. Uguccioni: «The chemokine network»

15th Annual International Antibody Engineering IBC's conference, San Diego CA, USA
E. Traggiai: «Cloning memory B cells to make human monoclonal antibodies»

XII Workshop Avances en Biologia Molecular por Jovenes Investigadores en el Extranjero, Madrid, Spain
A. Martín-Fontecha: «Induced recruitment of NK cells to lymph nodes provides IFN-gamma for Th1 priming»

2005

Keystone Symposium on Innate Immunity to Pathogens, Steamboat Springs, Colorado, USA
F. Sallusto: «Control of T Cell Immunity by Dendritic Cells»

MASIR 2005, Courmayeur, Italy
J. Geginat: «Chemokine receptor expression identifies pre-Th1, pre-Th2 and non-polarized cells among human $CD4^+$ central memory T cells»

SAFE-ID 10th Challenge in Virology Seminar, Saanen, Switzerland
A. Lanzavecchia: «Persistence of life-long immunity and vaccines»

Seminar at the Anatomic Institute, University of Bern, Switzerland
M. Manz: «Immunoreconstitution of mice and men»

European Dermatology Forum 2005, Interlaken, Switzerland
A. Lanzavecchia: «Exploring and Exploiting immunological memory»

Keystone Symposium on Dendritic Cells at the Centre of Innate and Adaptive Immunity: Eradication of Pathogens and Cancer and Control of Immunopathology, Vancouver, Canada, USA
F. Sallusto: «Human Dendritic Cells and their Role in Triggering T Cell Responses»

3rd EAACI-Davos Meeting, Basic Immunology in Allergy and Clinical Immunology, Davos, Switzerland
M. Manz: «Dendritic cell development from hematopoietic stem and progenitor cells»

Seminar at the University of Brescia, Brescia, Italy
M. Molinari: «Protein folding and quality control in the endoplasmic reticulum...and an approach to modulate production of the toxic amyloid-beta peptide»

Seminar at the University of Tübingen, Germany
J. Geginat: «Homeostasis of human memory T cell subsets»

Annual Congress of the Swiss Societies of Allergology and Immunology and of Pharmacology and Toxicology, Bern, Switzerland
A. Lanzavecchia: «Exploring and Exploiting immunological memory»

NCCR Neural Plasticity and Repair Symposium, Kartause Ittingen, Switzerland
M. Molinari: «Beta-site specific intrabodies to decrease and prevent generation of Alzheimer's Abeta peptide»

Keystone Symposium on Leukocyte Trafficking: Cellular and Molecular Mechanism, Taos, New Mexico, USA
F. Sallusto: «Regulation of dendritic cell and lymphocyte migration to lymph nodes»

Cellular Therapy 2005: 2nd International Symposium on the Clinical Use of Cellular Products, Regensburg, Germany
M. Manz: «Dendritic cell development-New options for targeted therapies?»

Keystone Symposium on B Cell Development, Function and Disease, Steamboat Springs, Colorado, USA
A. Lanzavecchia: «Maintenance of B Cell subsets»

3rd International Symposium on the Clinical Use of Cellular Products: Cellular Therapy 2005, Regensburg, Germany
A. Martín-Fontecha: «Induced recruitment of NK cells to lymph nodes provides IFN-gamma for Th1 priming»

AAI Annual Meeting Experimental Biology 2005, San Diego, CA, USA
A. Lanzavecchia: «Identifying and making use of human memory B cells»

Cell Biology Meets the Immune System: Molecular Aspects of Host Pathogen Interactions, International Titisee Conference, Titisee, Germany
M. Molinari: «Protein quality control in the endoplasmic reticulum»

I vaccini nella prevenzione delle malattie infettive, Convegno del Ministero della Difesa, Rome, Italy
A. Lanzavecchia: «Le cellule della memoria»

Danish Society of Immunology, Annual Meeting 2005. Copenhagen, Denmark
F. Sallusto: «Regulation of T cell immunity by dendritic cells»

Seminar at the Fox Chase Cancer Center, Philadelphia, PA, USA
A. Lanzavecchia: «Vaccination and immunological memory»

Seminar at the Department of Microbiology and Immunology, University of Erlangen, Germany
M. Manz: «Reconstitution of the human adaptive immune system in mice»

Giornata Europea dell'Immunologia, Università degli Studi di Milano, Milan, Italy
A. Lanzavecchia: «Memoria immunologica: dai modelli ai vaccini»

14th International Symposium on Calcium and Calcium Binding Proteins in Health and Disease, Banff, Canada, USA
F. Grassi: «Control of T cell activation by calreticulin»

Protein Maturation and Function, Oulu, Finland
M. Molinari: «EDEM and ERAD in protein folding and quality control»

Seminar at the University of Erlangen, Germany
J. Geginat: «Generation and maintenance of T cell memory»

Conference of the Swiss Society of Internal Medicine, EOC Lugano, Switzerland
A. Lanzavecchia: «Immunology and infectious diseases»

15th Nikolas Symposium on Langerhans Cell Histiocytosis, Athens, Greece
M. Manz: «Dendritic cell development-New options for targeted therapies?»

5th FOCIS Annual Meeting, Boston, Massachusetts, USA
A. Lanzavecchia: «Signals for T and B Cell Activation»

1st EMBL-Monterotondo PhD Symposium, Rome, Italy
M. Manz: «Immunoreconstitution of mice and men»

Gordon Research Conference on Viruses and Cells, Barga, Lucca, Italy
A. Lanzavecchia: «Vaccination and immunological memory»

ENII Conference 2005, 'Fundamentals of innate and adaptive immunity: basic advances and clinical potentials', Ile de Les Embiez, France
F. Sallusto: «Quantitative aspects in T cell priming and memory generation»
J. Geginat: «Regulation of IL-7R expression and IL-7 responsiveness in TCR-activated human T cells»

Seminar at the Department of Internal Medicine, Division of Infectious Diseases, The University of Texas Southwestern Medical Center, Texas, USA
M. Manz: «Human adaptive immune system reconstitution in mice»

9th International Conference on Malignant Lymphoma, Lugano, Switzerland
A. Lanzavecchia, Chairman session: «Immunotherapy»

4th National Conference SIICA, Brescia, Italy
F. Sallusto: «Generation and maintenance of effector and memory T lymphocytes»

Stages in Ematologia, 'Autoimmunità: meccanismi di azione e disordini clinici' Istituto di Ematologia e Oncologia Medica «L. e A. Seragnoli», University of Bologna, Italy
F. Sallusto «Cellule dendritiche e autoimmunità»
M. Uguccioni. «Chemokines in autoimmunity»

National Institute of Allergy and Infectious Diseases (NIAID), Biodefence Workshop on humanized mice, Bethesda, Maryland, USA
M. Manz: «huAIS-RG mice»

First Symposium on Curative Cell Therapies for autoimmune diseases, Berlin, Germany
F. Sallusto «Generation and maintenance of T cell memory»

BIO 2005, Philadelphia, PA, USA
A. Lanzavecchia: «B Cells Never Forget: Novel approaches to antibody generation and antigen discovery»

7th Annual Sabin Colloquium on Cancer Vaccines and Immunotherapy, Cold Spring Harbor, Long Island, NY, USA
A. Lanzavecchia: «Vaccination and immunological memory»

Stichting Ontwikkelingsfonds Immunohaematologie «The Dageraad Symposium 2005», Dageraad, The Netherlands
F. Sallusto «Control of T cell immunity by dendritic cells»

Protein Folding and Transport in Health and Disease, FEBS-IUBMB Meeting, Bucharest, Romania
M. Molinari: «EDEM and ERAD in protein folding and quality control»

WAC 2005, Munich, Germany
A. Lanzavecchia: «T cell fitness»

Publications

2000-2005

1. **Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization**
Cella M., Facchetti F., Lanzavecchia A., and Colonna M.
Nat Immunol 2000; 1:305-310

2. **The role of aquaporins in dendritic cell macropinocytosis**
de Baey A. and Lanzavecchia A.
J Exp Med 2000; 191:743-748

3. **A shift in the phenotype of melan-A-specific CTL identifies melanoma patients with an active tumor-specific immune response**
Dunbar P. R., Smith C. L., Chao D., Salio M., Shepherd D., Mirza F., Lipp M., Lanzavecchia A., Sallusto F., Evans A., Russell-Jones R., Harris A. L., and Cerundolo V.
J Immunol 2000; 165:6644-6652

4. **Cholera toxin induces maturation of human dendritic cells and licences them for Th2 priming**
Gagliardi M. C., Sallusto F., Marinaro M., Langenkamp A., Lanzavecchia A., and De Magistris M. T.
Eur J Immunol 2000; 30:2394-2403

5. **Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells**
Langenkamp A., Messi M., Lanzavecchia A., and Sallusto F.
Nat Immunol 2000; 1:311-316

6. **Cutting edge: recombinase-activating gene expression and V(D)J recombination in CD4+CD3low mature T lymphocytes**
Lantelme E., Palermo B., Granziero L., Mantovani S., Campanelli R., Monafo V., Lanzavecchia A., and Giachino C.
J Immunol 2000; 164:3455-3459

7. **From synapses to immunological memory: the role of sustained T cell stimulation**
Lanzavecchia A. and Sallusto F.
Curr Opin Immunol 2000; 12:92-98

8. **Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells**
Lanzavecchia A. and Sallusto F.
Science 2000; 290:92-97

9. **Dendritic cell maturation is induced by mycoplasma infection but not by necrotic cells**
Salio M., Cerundolo V., and Lanzavecchia A.
Eur J Immunol 2000; 30:705-708

10. **Understanding dendritic cell and T-lymphocyte traffic through the analysis of chemokine receptor expression**
Sallusto F. and Lanzavecchia A.
Immunol Rev 2000; 177:134-140

11. **The role of chemokine receptors in primary, effector, and memory immune responses**
Sallusto F., Mackay C. R., and Lanzavecchia A.
Annu Rev Immunol 2000; 18:593-620

12. **Functional subsets of memory T cells identified by CCR7 expression**
Sallusto F., Langenkamp A., Geginat J., and Lanzavecchia A.
Curr Top Microbiol Immunol 2000; 251:167-171

13. **Macrophages infiltrating the tissue in chronic pancreatitis express the chemokine receptor CCR5**
Goecke H., Forssmann U., Uguccioni M., Friess H., Conejo-Garcia J. R., Zimmermann A., Baggiolini M., and Buchler M. W.
Surgery 2000; 128:806-814

14. **Human chondrocytes express functional chemokine receptors and release matrix-degrading enzymes in response to C-X-C and C-C chemokines**
Borzi R. M., Mazzetti I., Cattini L., Uguccioni M., Baggiolini M., and Facchini A.
Arthritis Rheum 2000; 43:1734-1741

15. **Constitutive expression of stromal derived factor-1 by mucosal epithelia and its role in HIV transmission and propagation**
Agace W. W., Amara A., Roberts A. I., Pablos J. L., Thelen S., Uguccioni M., Li X. Y., Marsal J., renzana-Seisdedos F., Delaunay T., Ebert E. C., Moser B., and Parker C. M.
Curr Biol 2000; 10:325-328

16. **Signal transduction by CXC chemokine receptor 4. Stromal cell-derived factor 1 stimulates prolonged protein kinase B and extracellular signal-regulated kinase 2 activation in T lymphocytes**
Tilton B., Ho L., Oberlin E., Loetscher P., Baleux F., Clark-Lewis I., and Thelen M.
J Exp Med 2000; 192:313-324

17. **Evidence for a pool of coronin in mammalian cells that is sensitive to PI 3-kinase**
Didichenko S. A., Segal A. W., and Thelen M.
FEBS Lett 2000; 485:147-152

18. **Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production**
Breitfeld D., Ohl L., Kremmer E., Ellwart J., Sallusto F., Lipp M., and Forster R.
J Exp Med 2000; 192:1545-1552

19. **BDCA-2, a novel plasmacytoid dendritic cell-specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon alpha/beta induction**
Dzionek A., Sohma Y., Nagafune J., Cella M., Colonna M., Facchetti F., Gunther G., Johnston I., Lanzavecchia A., Nagasaka T., Okada T., Vermi W., Winkels G., Yamamoto T., Zysk M., Yamaguchi Y., and Schmitz J.
J Exp Med 2001; 194:1823-1834

20. **Cytokine-driven proliferation and differentiation of human naive, central memory, and effector memory CD4(+) T cells**
Geginat J., Sallusto F., and Lanzavecchia A.
J Exp Med 2001; 194:1711-1719

21. **Mouse pre-immunocytes as non-proliferating multipotent precursors of macrophages, interferon-producing cells, CD8alpha(+) and CD8alpha(-) dendritic cells**
Bruno L., Seidl T., and Lanzavecchia A.
Eur J Immunol 2001; 31:3403-3412

22. **Specialization and complementarity in microbial molecule recognition by human myeloid and plasmacytoid dendritic cells**
Jarrossay D., Napolitani G., Colonna M., Sallusto F., and Lanzavecchia A.
Eur J Immunol 2001; 31:3388-3393

23. **Molecular regulation of CC-chemokine receptor 3 expression in human T helper 2 cells**
Scotet E., Schroeder S., and Lanzavecchia A.
Blood 2001; 98:2568-2570

24. **Exploring pathways for memory T cell generation**
Sallusto F. and Lanzavecchia A.
J Clin Invest 2001; 108:805-806

25. **Regulation of T cell immunity by dendritic cells**
Lanzavecchia A. and Sallusto F.
Cell 2001; 106:263-266

26. **The instructive role of dendritic cells on T cell responses: lineages, plasticity and kinetics**
Lanzavecchia A. and Sallusto F.
Curr Opin Immunol 2001; 13:291-298

27. **Antigen decoding by T lymphocytes: from synapses to fate determination**
Lanzavecchia A. and Sallusto F.
Nat Immunol 2001; 2:487-492

28. **Migration and function of antigen-primed nonpolarized T lymphocytes in vivo**
Iezzi G., Scheidegger D., and Lanzavecchia A.
J Exp Med 2001; 193:987-993

29. **Organization of plasma membrane functional rafts upon T cell activation**
Tuosto L., Parolini I., Schroder S., Sargiacomo M., Lanzavecchia A., and Viola A.
Eur J Immunol 2001; 31:345-349

30. **Antigen processing and recognition**
Cresswell P. and Lanzavecchia A.
Curr Opin Immunol 2001; 13:11-12

31. **Eotaxin is a natural antagonist for CCR2 and an agonist for CCR5**
Ogilvie P., Bardi G., Clark-Lewis I., Baggiolini M., and Uguccioni M.
Blood 2001; 97:1920-1924

32. **CC chemokines and the receptors CCR3 and CCR5 are differentially expressed in the nonneoplastic leukocytic infiltrates of Hodgkin disease**
Buri C., Korner M., Scharli P., Cefai D., Uguccioni M., Mueller C., Laissue J. A., and Mazzucchelli L.
Blood 2001; 97:1543-1548

33. **Cell cycle-dependent expression of CXC chemokine receptor 3 by endothelial cells mediates angiostatic activity**
Romagnani P., Annunziato F., Lasagni L., Lazzeri E., Beltrame C., Francalanci M., Uguccioni M., Galli G., Cosmi L., Maurenzig L., Baggiolini M., Maggi E., Romagnani S., and Serio M.
J Clin Invest 2001; 107:53-63

34. **Dancing to the tune of chemokines**
Thelen M.
Nat Immunol 2001; 2:129-134

35. **Rapid inactivation of stromal cell-derived factor-1 by cathepsin G associated with lymphocytes**
Delgado M. B., Clark-Lewis I., Loetscher P., Langen H., Thelen M., Baggiolini M., and Wolf M.
Eur J Immunol 2001; 31:699-707

36. **Is dimerization of chemokine receptors functionally relevant?**
Thelen M. and Baggiolini M.
Sci STKE 2001; 2001:E34-

37. **Carboxyl-terminal modulator protein (CTMP), a negative regulator of PKB/Akt and v-Akt at the plasma membrane**
Maira S. M., Galetic I., Brazil D. P., Kaech S., Ingley E., Thelen M., and Hemmings B. A.
Science 2001; 294:374-380

38. **Rac and phosphatidylinositol 3-kinase regulate the protein kinase B in Fc epsilon RI signaling in RBL 2H3 mast cells**
Djouder N., Schmidt G., Frings M., Cavalie A., Thelen M., and Aktories K.
J Immunol 2001; 166:1627-1634

39. **Kinetics of GATA-3 gene expression in early polarizing and committed human T cells**
Lantelme E., Mantovani S., Palermo B., Campanelli R., Sallusto F., and Giachino C.
Immunology 2001; 102:123-130

40. **Expression and immunogenicity of oncofetal antigen-immature laminin receptor in human renal cell carcinoma**
Zelle-Rieser C., Barsoum A. L., Sallusto F., Ramoner R., Rohrer J. W., Holtl L., Bartsch G., Coggin J. H., Jr., and Thurnher M.
J Urol 2001; 165:1705-1709

41. **Two waves of nuclear factor kappaB recruitment to target promoters**
Saccani S., Pantano S., and Natoli G.
J Exp Med 2001; 193:1351-1359

42. **Origin and migratory properties of dendritic cells in the skin**
Sallusto F.
Curr Opin Allergy Clin Immunol 2001; 1:441-448

43. **Chemoattractants MDC and TARC are secreted by malignant B-cell precursors following CD40 ligation and support the migration of leukemia-specific T cells**
Ghia P., Transidico P., Veiga J. P., Schaniel C., Sallusto F., Matsushima K., Sallan S. E., Rolink A. G., Mantovani A., Nadler L. M., and Cardoso A. A.
Blood 2001; 98:533-540

44. **Phosphatidylinositol 3-kinase c2alpha contains a nuclear localization sequence and associates with nuclear speckles**
Didichenko S. A. and Thelen M.
J Biol Chem 2001; 276:48135-48142

45. **Maintenance of serological memory by polyclonal activation of human memory B cells**
Bernasconi N. L., Traggiai E., and Lanzavecchia A.
Science 2002; 298:2199-2202

46. **A temporal and spatial summation model for T-cell activation: signal integration and antigen decoding**
Rachmilewitz J. and Lanzavecchia A.
Trends Immunol 2002; 23:592-595

47. **Progressive differentiation and selection of the fittest in the immune response**
Lanzavecchia A. and Sallusto F.
Nat Rev Immunol 2002; 2:982-987

48. **TCR-independent proliferation and differentiation of human CD4+ T cell subsets induced by cytokines**
Geginat J., Campagnaro S., Sallusto F., and Lanzavecchia A.
Adv Exp Med Biol 2002; 512:107-112

49. **Optimizing anti-CD3 affinity for effective T cell targeting against tumor cells**
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