Institute for Research in Biomedicine

Annual Report 2003

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RESEARCH REPORTS

1. Protein folding and quality control in the endoplasmic reticulum

Student: Klara Kristin Eriksson
Researchers: Carmela Galli, Maurizio Molinari
Technician: Verena Calanca-Piccaluga

Calreticulin (Crt) and calnexin (Cnx) are homologous lectins that serve as molecular chaperones for glycoproteins in the endoplasmic reticulum of eukaryotic cells. To learn more about distinct and shared functions of Crt and Cnx in the endoplasmic reticulum (ER), we followed folding and secretion of a variety of glycoproteins in cells devoid of one or the other of these lectins, as well as under conditions in which binding to both lectins was inhibited. We found that Crt-depletion specifically accelerated the maturation of cellular and viral glycoproteins with a modest decrease in folding efficiency. Cnx-depletion prevented proper maturation of some proteins such as influenza hemagglutinin but did not interfere appreciably with the maturation of several others. 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 Crt and Cnx was prevented. Although not fully interchangeable during assistance of glycoprotein folding, Crt and Cnx may therefore work, independently, as efficient and crucial factors for retention in the ER of non-native polypeptides.

Publications n. 103, 113

2. The role of ER lectins in ER-associated protein degradation (ERAD)

Researchers: Carmela Galli, Maurizio Molinari
Technician: Verena Calanca-Piccaluga

Terminally misfolded glycoproteins are dislocated into the cytosol and degraded by the proteasome in processes collectively defined as ER-associated degradation (ERAD). The importance of unravelling the mechanisms of ERAD in mammalian cells is emphasized by the recent description of the decisive role played by this process in hereditary human *conformational diseases* (e.g., cystic fibrosis and α1-antitrypsin deficiency leading to lung emphysema). We are determining the molecular mechanisms of ERAD by monitoring degradation of several variants of the pancreatic isoform of human beta secretase (BACE457, (Molinari et al. 2002)). We found that upon release from Cnx, misfolded BACE457 formed relatively small disulfide-bonded complexes as physiologic intermediates of the degradation process. These covalent complexes contained the lumenal chaperones BiP and PDI and accumulated in the ER upon proteasome inhibition. They were efficiently disentangled to allow BACE dislocation and degradation. Our data also revealed how unproductive folding attempts are terminated. Overexpression of EDEM, a putative ER-resident mannose-binding lectin, accelerated degradation of BACE by promoting the transfer of the ERAD substrate from the folding cycle governed by Cnx to the preparative phase for dislocation governed by BiP and PDI. Reduction of the lumenal level of EDEM prolonged association of ERAD candidates with Cnx thereby delaying their disposal.

Publication n. 083, 114

3. Folding, quality control and degradation of secretory proteins in cells depleted of the transcription factor Xbp1

Student: Klara Kristin Eriksson
Researchers: Carmela Galli, Maurizio Molinari
Technician: Verena Calanca-Piccaluga

A stringent quality control selects misfolded polypeptides generated in the ER for destruction. We have shown that variation in the intralumenal level of the ER-resident lectin EDEM affects the rate of glycoprotein degradation from the ER. EDEM is an unfolded protein response (UPR)-regulated gene and its intralumenal level is controlled by the stress-regulated transcription factor Xbp1. We therefore determined if cells depleted of Xbp1 had aberrant capacity to degrade misfolded glycoproteins and we determined if these cells were able to assist protein folding and to maintain production of high amount of secretory proteins. Our results showed that depletion of Xbp1 affected stress-induced activation of EDEM and of several other ER chaperones (Cnx, UDP-glucose: glycoprotein glucosyltransferase, ERp29). As a consequence, Xbp1-depleted cells showed impaired capacity to degrade glycoproteins. When these cells were converted into factories for production of human beta-secretase, maturation of this N-glycosylated aspartic protease progressed normally at first. However, failure to accomplish efficiently ERAD led to slow accumulation of side-products of beta-secretase biosynthesis that progressively compromised the capacity of Xbp1-depleted cells to assist protein folding and secretion efficiently. Potentiation of the ERAD machinery (EDEM up-regulation) prevented luminal accumulation of misfolded beta-secretase, thus sustaining protein folding in Xbp1-depleted cells. Potentiation of the folding machinery (Cnx up-regulation) had on the other hand no beneficial consequence on the capacity of these cells to maintain high secretory capacity. These findings underscore the crucial role of ERAD, and in particular of the regulation of the intralumenal level and activity of EDEM in coupling disposal of misfolded side-products of protein biosynthesis to maintenance of efficient maturation and quality control of secretory proteins in the mammalian ER.

4. Kifunensine affects differentiation of B cells into plasma cells

Students: Klara Kristin Eriksson, Riccardo Vago
Supervisor: Maurizio Molinari

The transcription factor XBP1 (required for efficient ERAD, project 3) is required for the multi-step process in which stimulated B-lymphocytes proliferate and differentiate into antibody secreting plasma cells (PCs). Activated B cells develop a highly expanded ER to prepare for the massive load of immunoglobulin (Ig) produced upon terminal differentiation. In this study we investigated the effect of kifunensine (Kif) on the process of B cell differentiation into PCs. Kif is an inhibitor of α-mannosidases that have been shown to block ERAD of glycoproteins. We found that Kif interfered with B cell differentiation into PCs. Already 2 days after polyclonal stimulation, Kif-treated naïve B cells expressed an increased level of transcripts encoding proteins involved in the unfolded protein response (UPR) and ERAD, showing that a premature UPR, most likely caused by accumulation of proteins in the ER when ERAD was inhibited, was triggered in these cells.

Five days after stimulation, very few terminally differentiated PCs were found in Kif-treated cultures of stimulated naïve B cells as determined by surface marker expression, Ig secretion and level of transcripts of genes involved in terminal differentiation. Moreover, we found that Kif reduced total Ig production and caused intracellular accumulation of detergent-insoluble Ig in an IgG1-producing EBV immortalized B cell clone, supporting the role of α-mannosidase function in ERAD. These results suggest that Kif could both affect adaptation during B cell differentiation by interfering with normal transcriptional control of this process, and interfere with maintenance of secretory capacity of terminally differentiated cells. Taken together, this indicates that adaptation to increased cargo load is essential for generation and survival of cells with high secretory capacity.

5. Protein aggregation as an intermediate step in ERAD

Student: Silvia Olivari
Supervisor: 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.

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

Researchers: Carmela Galli, 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-glycan 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.

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

Student: Tatiana Soldà
Supervisor: 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) super family. ERp57 specifically promotes the oxidative folding of newly synthesized glycoproteins. The aim of this work is to determine consequences of ERp57 down-regulation 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 and the antisense oligonucleotides connected by a short loop. These cells will now be used to monitor oxidative folding of model glycoproteins such as influenza hemagglutinin and beta secretase.

8. An unfolded protein response to down-regulation of Cnx and ERp57 decides on cell death or adaptation

Student: Klara Kristin Eriksson
Supervisor: Maurizio Molinari

RNA interference, i.e. sequence specific silencing of gene expression, is used to assess consequences of target protein down-regulation. We designed small interfering RNAs, i.e. duplexes of 21-nt RNAs, which selectively lowered the intracellular level of Cnx and ERp57 in HeLa cells. We assessed consequences of reduced level of Cnx and ERp57 on pathways that regulate ER homeostasis and cell death. Down-regulation of Cnx caused a general reduction of transcription and massive cell death. Transcription of the gene encoding CHOP, a protein involved in apoptosis, was not affected. Down-regulation of ERp57 triggered a moderate UPR, characterized by splicing of XBP1 and up-regulation of BiP and Ero-1Lβ. Reduced cell death was observed compared with cells down-regulating Cnx. We hypothesize that moderate induction of UPR in cells with reduced level of ERp57 protects from ER stress-induced cell death.

9. An alternative approach to regulate proteolytic processing of the amyloid precursor protein and inhibit the generation of the amyloid-beta peptide *in vivo*

Researchers: Carmela Galli, Maurizio Molinari
Technician: Verena Calanca-Piccaluga

Proteolytic cleavage of theβ-amyloid precursor protein (APP) by β- and γ-secretases generates a highly hydrophobic peptide, the amyloid β-peptide (Aβ, which aggregates to form oligomers and fibers. Deposition of these fibers initiates a variety of toxic insults leading to the vast neurodegenerative processes observed in Alzheimer's disease (AD) patient's brains. Since a major risk factor for AD is aging, one can expect a sharp increase in the number of patients in the near future. For that reason, therapeutic treatment against this devastating disease is urgently required. One possibility is given by immunotherapy

based on injection of pre-aggregated synthetic Aβ. This may elicit generation of antibodies against plaques. When this happens, patients show a stabilization of the cognitive abilities compared to rapidly worsening control patients. Unfortunately, side effects such as meningoencephalitis and infiltration of white matter with macrophages have emerged in some of the patients and clinical trials have been momentarily stopped. Another approach is based on designing selective secretase inhibitors because β is generated upon β- and γ-secretase-mediated cleavage of APP. U γ-secretase is required for Notch and other signaling pathways and specific inhibitors have shown devastating side-effects when employed *in vivo*. β-secretase inhibitors are more likely to work because β-secretase does not seem to be an essential gene. However, the wide and complex active site of the enzyme makes development of small-molecule inhibitors penetrating the brain challenging. We would like to propose an alternative approach that we define as *intracellular vaccination* aiming in reducing the cellular production of Ab. The main purpose of this project is to discover and develop molecules such as micro-antibodies that bind specifically to APP *in vivo* and divert it away from the amyloidogenic proteolytic processing pathway.

10. Regulation of the inflammatory transcription factor NF-kB *in vivo*

Student: Ivan Marazzi
Supervisor: Gioacchino Natoli
Researchers: Simona Saccani, Daniela Bosisio

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 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.

Publications n. 031, 050, 096, 112

11. Transcriptional repression and termination in the inflammatory response

Researchers: Serafino Pantano, Simona Saccani, Gioacchino Natoli

A few transcriptional repressors are rapidly down-regulated following DC stimulation with bacterial products. The hypothesis we are testing is that rapid down-regulation of transcriptional repressors may be permissive for the induction of a subset of rapidly induced genes. We have found that the transcriptional repressor BCL-6 is the target of an as yet unidentified inflammatory pathway triggering its phosphorylation and rapid degradation. Further characterization of the intermediates of this pathway is currently undergoing. Consistent with this working model, we have also found that several inflammatory genes in DCs are associated with a repressive histone modification (namely methylation of histone H3 at Lys 9) that is erased upon activation and then subsequently restored, concurrently with post-induction transcriptional repression.

Publication n. 063

12. CCR2-induced RhoGTPase activation

Researchers: Sylvia Thelen, Marcus Thelen

Activation of the small RhoGTPases downstream of chemokine receptors is essential for cell migration. The large number of guanine exchange factors (GEF) is widely interpreted as indication that these proteins are responsible for the spatiotemporal regulation of RhoGTPase. Recently, a novel GEF, P-Rex1, was shown to be activated by the βγ-subunits of heterotrimeric G-proteins and the Pi 3-kinase products PIP_3. We have fused P-Rex to green fluorescent protein (GFP) and expressed the construct in mouse pre-B cells. Fluorescence microscopy indicates that P-Rex is recruited to sites of transient actin polymerization, such as lamelipodia of stimulated cells, indicating that this GEF could mediate chemokine receptor mediated activation of RhoGTPases during chemotaxis. The aim of the study is to elucidate the biochemical events that regulate the localization of P-Rex and identify mechanism that activate its activity as exchange factor.

13. Cellular functions of the class II HsPI3K-C2α

Researchers: Svetlana A. Didichenko, Marcus Thelen

The class II HsPI3K-C2α in quiescent and proliferating cells becomes phosphorylated. Stress-dependent and mitotic phosphorylation of HsPI3K-C2α occurs on the same serine residue, Ser259, within a recognition motif for proline-directed kinases. Mitotic phosphorylation of HsPI3K-C2α can be attributed to cdc2 activity, and that stress-induced phosphorylation occurs via JNK/SAPK. Mitotic phosphorylation of HsPI3K-C2α provides an essential signal for proteosome-dependent degradation of the protein at the M/G1 transition of the cell cycle. Over expression of HsPI3K-C2α and of defined domains of the protein affect the centrosomal structure and the normal progression through M phase of the cell cycle. Consistent with the localization of the kinase at the interphase centrosomes and its phosphorylation at the centrosomes and subsequent degradation during mitosis, suggest that HsPI3K-C2α is part of a checkpoint control in M phase.

14. Characterization of the putative chemokine receptor RDC1

Student: Simona Infantino
Supervisor: Marcus Thelen

RDC1 is a putative chemokine receptor based on its seven transmembrane domain structure and its homology to CXC-chemokine receptors. The receptor maps to mouse chromosome 1 and human chromosome 2 (2q37.3) where also the genes of CXCR4 and CXCR2 are found. RDC1 is highly conserved among species (Xenopus, rodents, dog and humans) and like CXCR4 can function as HIV-co-receptor. We have generated monoclonal antibodies against human RDC1. Immunohistochemical analysis and in situ hybridization reveals expression of the receptor is in subsets of lymphocytes in secondary lymph follicles. Cell lines that stably express RDC1 were prepared to search for potential ligands in culture supernatants of lymphatic tissue.

15. Chemokine receptor mediated signal transduction

Researchers: Sylvia Thelen, Marcus Thelen

We have shown that following stimulation with MCP-1 and eotaxin the chemokine receptor CCR2 activates ERKs using different signal transduction pathways. Activation of the MAPK cascade by eotaxin is essential for the antagonistic effect of the chemokine towards functional responses elicited with MCP-1. Signal transduction elicited by eotaxin does not lead to G-protein activation but depends on binding of CCR2 to G_i-proteins. The data support the hypothesis that CCR2 can assume different ligand-induced receptor active states. In line with such view type IB and type IA PI 3-kinases become activated with MCP-1 and eotaxin, respectively. The aim of the project is to disclose the molecular environment of CCR2 to understand the mechanisms of distinct signal transduction.

16. Stimulation of chemotaxis by the chemokine receptor CXCR4

Student: Elena Palmesino
Supervisor: Marcus Thelen

Expression of the chemokine receptor CXCR4 is essential for bone marrow retention and maturation of B cells as well as organ development during embryogenesis. We observed that human B-cell lines representing different stages of B-cell maturation, express functional CXCR4, as measured by the activation of intracellular signal transduction pathways and receptor internalization, but progressively lose their capacity to migrate in response to CXCL12 (SDF-1). The signal transduction pathway(s) that are activated by chemokine receptors and leads to cell migration are poorly understood. It is generally assumed that RhoGTPases are key regulators for cytoskeletal rearrangements during cell migration stimulated by different receptor systems. Small GTPases can be kept in an inactive state by GDI-proteins (GTP dissociation inhibitors) and are activated by GEF's (GTP exchange factors) and are deactivated by GAP (GTPase activating proteins). We investigated chemokine receptor-mediated activation of RhoGTPases and found that during maturation B cells lose the ability to activate RhoGTPases. We speculate that CXCR4 couples differently to downstream effectors which lead to RhoGTPase activation. We have therefore established an immunoprecititation protocol using conformation sensitive antibodies to determine the proteome of CXCR4 by mass spectrometry.

17. The role of Z gene product in T cell development and function
Student: Zuzana Garajova
Supervisor: Klaus Karjalainen

The novel Z protein is specifically expressed in thymocytes after T cell commitment and in all subsequent T cell developmental stages. We have produced monoclonal antibodies against Z in order to study its intracellular localization as well as to identify potential interacting protein partners. Mice deficient in Z are being generated by gene targeting to gain further insights of its biological role in the T lineage cells.

18. The role of a novel home box containing transcription factor in lymphoid cells
Student: Piotr Tetlak
Supervisor: Klaus Karjalainen

By screening subtractive cDNA libraries we have identified a novel transcription factor X that is strongly expressed in thymocytes and detectably also in mature T and B cells. The gene targets of X are being defined by using an inducible system based on fusions of X DNA binding domain to repressor or activator domains followed by microarray analysis. At the same time gene targeted mice are being produced in order to further advance of our understanding of X.

19. The function of Lag 3 in immunecytes of non T cell origin
Student: Malgorzata Kisielow
Supervisor: Klaus Karjalainen

With a new panel of monoclonal antibodies we have found that Lag3 is not only expressed on activated T cells but also in inducible manner on B cells as well as on myeloid dendritic cells. Mechanisms of induction and possible functional consequences of inducible Lag3 expression in these cells are being investigated.

20. R3H dependent and independent phosphorylation of TARPP
Researchers: Jan Kisielow, Klaus Karjalainen

TARPP is a thymocyte specific splice variant of the ARPP-21 protein. At mRNA level both isoforms are expressed in the thymus and brain, but the proteins are specifically expressed in the thymus (TARPP) or brain (ARPP-21). TARPP is tightly regulated during development. It appears in early precursors together with the commitment to the T cell lineage (TCR gene rearrangement) and is downregulated at the CD4 CD8 double-positive stage. The downregulation of TARPP is triggered by the TCR engagement during positive selection. The locus encoding TARPP gives rise to many protein isoforms generated by differential splicing, including the short 21kDa protein called ARPP-21 and TARPP isoforms A, B, C, D and E. TARPP is a 100kDa cytoplasmic protein that contains two evolutionally conserved domains (R3H and EREE) and is homologous to putative human proteins KIA1002, KIA0029 and to the *Drosophila* protein Encore. A proposed role for the R3H domain is binding to single-stranded nucleic acids. It is present in more then 100

proteins from Eubacteria to mammals. R3H and EREE domains are located in a region of high homology that defines a novel family of proteins with unknown function. However one of the family members, Encore is implicated in the control of the protein levels of Gurken, which is involved in *Drosophila* oogenesis, suggesting a role for these proteins in RNA metabolism and/or translational control. In order to understand TARPP function and the contribution of the R3H domain cell lines over-expressing different TARPP isoforms with an intact or mutated R3H domain were generated and analyzed. Our data indicates R3H domain function in TARPP phosphorylation and suggests a role for TARPP in the regulation of cell survival.

21. Subcellular routing of signals required for pre-T cell development

Student: Denise Ferrera
Supervisor: Fabio Grassi

Exit from the endoplasmic reticulum and partition of the pre-TCR into glycolipid-enriched membrane domains (rafts) together with the $p56^{lck}$ Src kinase is sufficient to initiate pre-TCR signaling without any need for ligation. In line with this property of signalling in a ligand-independent fashion the pre-TCR is constitutively routed to lysosomes after reaching the cell surface. The signalling properties of the pre-TCR are mimicked by cross-linking of the clonotype-independent complex (CIC) expressed on the cell surface of pre-T cell progenitors and constituted by calnexin (CNX) in noncovalent association with CD3. Indeed, we could detect a fraction of CICs in rafts of a SCID thymocyte derived cell line (SCIET.27). To analyze the molecular requirements of CIC to generate "pre-TCR like" signalling we transfected SCIET.27 cells 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. Analysis of signal transduction by CIC mutated in the cytoplasmic tail of calnexin with rafts partition in the absence of endocytosis will be informative on the role of rafts versus endocytosis in pre-TCR signaling. *In vivo* tumorigenesis by mutated calnexins in a murine model of pre-TCR dependent leukemia/lymphoma will allow dissection of the role played by rafts versus endocytosis in leukemogenesis.

22. Role of calreticulin in T cell homeostasis

Student: Simona Porcellini
Supervisor: Fabio Grassi

Calreticulin (CRT) deficiency in mice is embryonically lethal because of altered cardiac development. In the absence of CRT cells display impaired inositol 1,4,5-trisphosphate (IP_3)-dependent Ca^{2+} release, which results in inefficient nuclear translocation of nuclear factor of activated T cell (NFAT). Indeed, sustained Ca^{2+} release from the endoplasmic reticulum is required to activate calcineurin phosphatase activity, which dephosphorylates NFAT allowing its nuclear targeting and gene regulation. Since NFAT has a central role in regulating T cell functions, we wanted to investigate whether CRT deficiency have an impact on lymphoid homeostasis. Then, we reconstituted recombinase-2-deficient (RAG-2)/common γ chain double knock-out (DKO) mice with fetal liver hemopoietic progenitors (FLP) from *crt*$^{-/-}$ embryos. RAG/γ chain DKO mice reconstituted with *crt*$^{-/-}$ FLP display phenotypic traits compatible with immunopathological damage of the skin and the eye starting at week 7 after reconstitution with some mice progressing to a wasting disease later on. β selection of immature thymocyte (CD25 and CD44 expression in the $CD4^-8^-$ double negative compartment as well as transition to the $CD4^+8^+$ double positive stage) and positive selection of $CD4^+8^+$ cells (analysed by upregulation of TCRαβ and CD69 expression) revealed no differences among the *crt*$^{-/-}$ chimera and the wildtype counterpart. Thus, in spite of involvement of calcium signaling and NFAT nuclear translocation at these T cell developmental transitions CRT seems dispensable for their accomplishment. Analysis of peripheral lymphoid organs revealed the presence in both the CD4 and CD8 αβ T cell lineages of an increased number of cells displaying markers of activation and constitutively secreting cytokines. Furthermore, *in vitro* activation of T cells from spleens of *crt*$^{-/-}$ chimeras resulted in bystander B cell proliferation with transition to plasma cell and immunoglobulin secretion in the culture medium. These phenomena could derive from inefficient deletion of autoreactive T cells in the thymus; however sensitivity to apoptosis by anti-CD3 treatment of thymocytes was unaltered with respect to *crt*$^{+/+}$ cells. These evidences suggest a critical role of CRT in the homeostasis of the peripheral T cell pool by negatively modulating the effector phase of the T cell immune response.

23. Reconstitution of a human adaptive immune system in CD34+ cord blood cell transplanted mice

Students: Laurie Chicha, Roxane Tussiwand
Supervisor: Markus G. Manz
Researchers: Elisabetta Traggiai, Antonio Lanzavecchia

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, sustain only limited development and maintenance of human lymphoid cells and rarely produce immune responses. We now have shown that intrahepatic injection of CD34+ human cord blood cells into conditioned newborn Rag2gc-/- mice leads to de novo development of B, T, dendritic (DC) and natural interferon producing cells (IPCs); formation of structured primary and secondary lymphoid organs; and production of functional immune responses. Using this model, we are now studying in more detail a) human T cell differentiation and selection, b) human in vivo DC and IPC differentiation, c) maintenance and differentiation of human hematopoietic stem and

progenitor cells in bone marrow of transplanted mice, and d) immune responses to EBV and HIV in vivo (studies on EBV are done in collaboration with Prof. Dr. J.C. Piffaretti and Prof. Dr. A. Rickinson, studies on HIV are done in collaboration with Dr. R. Speck and Prof. Dr. J. Frey). Further more, we will test if CD34+ cells from other sources as bone marrow and blood will be suitable to reconstitute mice with similar efficacy as seen from cord blood cells.

Publication n. 105

24. Human dendritic cell development

Students: Laurie Chicha, David Jarrossay
Supervisor: Markus G. Manz

In humans as in mice, dendritic cells (DCs) and natural interferon producing cells (IPCs) display different phenotypes, localizations, and functions. However, their lineal origins and critical developmental checkpoints have not been clarified. We recently identified human common myeloid progenitors (CMPs) and their downstream granulocyte/macrophage (GMPs) and megakaryocyte/erythrocyte progenitors (MEPs). In addition, we isolated candidate common lymphoid progenitor cells (CLPs). We are testing which of the restricted progenitors have DC and IPC developmental activity. We found that HSCs, CMPs, and GMPs are capable to generate large numbers of $CD11c^+$ DC in liquid cell culture. Using murine stroma cells (Ac6) and flt3-ligand, we have established an *in vitro* system for efficient development of both IPCs and DCs as well as B cells. This will enable us to identify human DC and IPC lineage origins and to directly compare the earliest genetic events involved in this differentiation process.

25. Flt3 tyrosin kinase regulation of dendritic cell development

Student: Roxane Tussiwand
Supervisor: Markus G. Manz
Researchers: Nobuyuki Onai, Aya Onai, Antonio Lanzavecchia

Throughout life dendritic cells are continuously generated from hematopoietic stem cells. This process must be tightly regulated, likely by homeostatic factors as cytokines and chemokines. We have shown that mouse DC can develop from early hematopoietic progenitor cells along a lymphoid and myeloid developmental pathway *in vitro* and *in vivo*. Therefore, DCs differentiation shows a developmental redundancy that is not observed for other cell types of the hematopoietic system. We are interested to evaluate what events are critical to maintain and drive or shut down the capacity of a given progenitor to develop into a DC. A candidate cytokine/receptor pair involved in this process is flt3-L/flt3. We found that flt3 expression is maintained in the hematopoietic hierarchy along both the lymphoid and myeloid DC developmental pathway from early progenitors to steady-state DC. In contrast, flt3 is not expressed in alternative developmental pathways that have lost DC potentials. To further evaluate its role in DC commitment, we are testing if artificial over-expression of flt3 in flt3-negative progenitors will rescue their DC developmental capacity.

26. *In vivo* depletion of dendritic cells-potential new methods for immunomodulation

Student: Roxane Tussiwand
Supervisor: Markus G. Manz
Researcher: Nobuyuki Onai

Dendritic and natural interferon-producing cell progenitors and their downstream steady-state cell populations express the flt3 receptor. F lt3-ligand$^{-/-}$ mice have massively reduced, and flt3-ligand-injected mice develop markedly increased numbers of both cell types. Thus, *in vivo* dendritic cell 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, we completely blocked dendritic and natural interferon-producing cell development in flt3-ligand supplemented (100 ng/ml) mouse bone marrow cell cultures, while dendritic cell development in GM-CSF supplemented (20 ng/ml) cultures was not affected. *In vivo* application 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. 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 will test this first in the setting of complete mismatched bone marrow transplantation.

27. Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming

Researchers: Alfonso Martín-Fontecha, Silvia Sebastiani, Mariagrazia Uguccioni, Antonio Lanzavecchia, Federica Sallusto

Antigen-pulsed dendritic cells (DC) are used as natural adjuvants for vaccination, but the factors that influence the efficacy of this treatment are poorly understood. We investigated the parameters that affect the migration of subcutaneously injected mouse mature DC to the draining lymph node. We found that the efficiency of DC migration varied with the number of injected DC and that CCR7$^{+/+}$ DC migrating to the draining lymph node, but not CCR7$^{-/-}$ DC that failed to do so, efficiently induced a rapid increase in lymph node cellularity, which was observed before the onset of T cell proliferation. We found also that DC migration could be increased up to 10 fold by pre-injection of inflammatory cytokines that increased the expression of the CCR7 ligand CCL21 in lymphatic endothelial cells. The magnitude and quality of CD4^{+} T cell response was proportional to the number of antigen-carrying DC that reached the lymph node and could be boosted up to 40-fold by pre-injection of TNF that conditioned the tissue for increased DC migration. These results indicate that DC number and tissue inflammation are critical parameters for DC-based vaccination. This work was done in collaboration with Martin Lipp and Uta E. Höpken, Max-Delbrück Center for Molecular Medicine, Berlin-Buch, Germany.

28. CCR7-independent recruitment of NK cells to stimulated lymph nodes provides IFN-γ for Th1 priming

Researchers: Alfonso Martín-Fontecha, Antonio Lanzavecchia, Federica Sallusto

Natural killer (NK) cells migrate to peripheral tissues where they act as effectors of innate immunity, but it is unclear under which conditions they can migrate to secondary lymphoid organs and participate in the induction of acquired immune responses. We found that NK cells were rapidly and transiently recruited into stimulated lymph nodes in a pertussis toxin-sensitive but CCR7-independent fashion. In stimulated but not control lymph node NK cells became activated and able to produce IFN-γ. NK cell recruitment was selectively induced by subcutaneous injection of mature dendritic cells (DC) and by some but not all adjuvants and correlated with induction of Th1 responses. Mice depleted of NK cells showed impaired Th1 differentiation, a defect that was corrected by transfer of IFN-$\gamma^{+/+}$, but not IFN-$\gamma^{-/-}$ NK cells. Furthermore, Th1 differentiation of TCR transgenic CD4 T cells was inefficient in IFN-$\gamma^{-/-}$ mice and transfer of IFN-$\gamma^{+/+}$ NK cells fully reconstituted Th1 polarization. These results reveal a novel pathway of NK cell migration and activation in antigen stimulated lymph nodes that provides an early source of IFN-γ required to enhance Th1 polarization. This work was done in collaboration with Lindy L. Thomsen and Sara Brett, Glaxo Smith and Klein, Stevenage, United Kingdom and Martin Lipp, Max Delbrück Center for Molecular Medicine, Berlin-Buch, Germany.

29. Synergistic activation of dendritic cells by TLR agonists

Researchers: Giorgio Napolitani, Antonio Lanzavecchia

Dendritic cells (DC) express a variety of Toll like receptors (TLR) which are selectively triggered by different microbial products. It has been recently shown that different agonists are capable of inducing responses of different magnitude and quality and these differences have been related to the type of adaptors utilized by each TLR. For instance, while all TLRs signal through the MyD88, TLR3 and TLR4 also recruit TRIF which triggers IFN-I production. In addition the strength and kinetics of signaling may differ among TLRs and depending on the concentration and type of agonist. We are interested to investigate whether different TLRs may synergize in DC activation. We tested the kinetics of MAP kinases and NF-kB activation and the extent of DC maturation and cytokine production in response to highly purified TLR agonists given alone or in different combinations, at different times and at various doses. We found that some but not all TLRs can potently synergize in the induction of cytokine production, especially IL-12 and IL-23. This synergism correlates with a sustained phosphorylation of c-Jun. The synergistic activation of DC explains how microbes that usually express several agonists are very potent stimuli for DC maturation and provides a new concept for the rationale design of adjuvants.

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

Researchers: Annalisa Macagno, Federica Sallusto

Microbial infections induce chemokine and cytokine cascades that coordinate innate immune defence. 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 dendritic cells, VB is a potent antagonist of proinflammatory stimuli acting through Toll Like Receptor 4 and CD40. Specifically, it inhibits activation of the MAP kinases JNK and p38 and of NF-kB, 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. This work is done in collaboration with Carlo Rossetti and Monica Molteni, University of Insubria, Varese, Italy and Siegfried Morath and Thomas Hartung, University of Konstanz, Konstanz, Germany.

31. ABC transporter activity discriminates human naive and memory B cells

Researchers: Stefan Wirths, Antonio Lanzavecchia

Human memory B cells can be identified as CD27+ cells expressing various levels of IgM or switched isotypes. We noticed however that highly purified CD27- B cells isolated from peripheral blood contained low numbers isotype switched B cells suggesting that they may contain a small proportion of memory B cells in disguise. We therefore searched for markers that may allow a better discrimination between human naïve and memory B cells. We found that R123, a vital dye that is extruded from cells via MDR1 is rapidly lost from naïve B cells (which are thus R123-) while it is retained in all isotype switched and CD27+ memory B cells (R123+). The subset of R123+IgG+ CD27- cells contains antigen specific B cells at frequencies comparable to those found in the conventional IgG+CD27+ subset, indicating that these are bona fide memory cells. To estimate the *in vivo* turnover of B cell subsets we measured the expression of Ki67, an antigen that is present in the nuclei of divided cells for approximately 3 days after mitosis. While only 0.05% of R123- naive B cells expressed Ki67, 4% and 3% of R123+CD27+ and R123+CD27- cells were Ki67+. These results are consistent with a continuous proliferation of memory B cells. We conclude that the presence of ABC transporters is a very powerful marker to discriminate between naïve and memory B cells. This difference may underlie a different susceptibility to some cytotoxic drugs.

32. Mechanisms that sustain serum antibody levels following vaccination
Researchers: Elisabetta Traggiai, Antonio Lanzavecchia

Following vaccination or natural infectious low levels of specific antibodies are maintained constant in serum for a human life time. Antibody levels can be sustained for some time by long lived plasma cells. In addition recent evidence from our and other laboratories suggests that plasma cells may be continuously generated from memory B cells via antigen-independent (polyclonal) mechanisms. To analyze the mechanisms that sustain serum antibody levels we primed and boosted healthy volunteers and analyzed the kinetics of serum antibodies and the frequency of antibody secreting cells and memory B cells. The primary response was slow, plasma cells secreting IgG antibodies appeared in blood from 16 to 30 days after immunization, concomitant with an elevation of serum antibody levels. In contrast the response to a booster immunization was much more prompt and transient. Circulating plasma cells reached very high numbers (up to 1% of PBMC) on day 6-7 but disappeared by day 16. Serum antibody levels increased up to 100 fold from day 6 to day 10, remained stable over a period of 3-6 weeks and decreased thereafter with a half life of 40 to 80 days until a constant level was reached 6 to 8 months after boosting. The constant level reached 8 months after boosting was higher than the pre-boost level and both levels correlated with the frequency of memory B cells. While the antibody kinetics was comparable in different individuals, there was a considerable variability in the parameters.
An equation that fits all the experimental curves was developed in collaboration with Roberto Puzone (University of Genoa, Genoa, Italy). The variables of this equation are the numbers of short lived and long lived plasma cells, their lifespan, the half-life of serum antibodies and the frequency of memory B cells. Based on these results and their modeling 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 lived 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.

33. An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS-coronavirus
Researchers: Elisabetta Traggiai, 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. 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 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, Kanta Subbarao, NIAID, Bethesda, and Rino Rappuoli, Chiron Vaccines, Siena.

Publication n. 110

34. A role for innate immunity in human naïve B cell activation

Student: Claudia Ruprecht
Supervisor: Antonio Lanzavecchia

We used highly purified naïve B cells to analyze the stimuli that are necessary for their proliferation, switch and differentiation to Ig-secreting cells. Triggering of the BCR by $F(ab')_2$ fragments of anti-Ig antibodies in the presence of cognate T cell help (mediated by TSST) was sufficient to induce initial proliferation of naïve B cells, but the proliferating cells died by day 5. Addition of agonists of TLR2, TLR7 and TLR9 (i.e. TLRs which we have previously shown to be rapidly up-regulated following BCR triggering) sustained the proliferative response and led to the accumulation of large number of activated B cells and IgM-secreting cells by day 7. Switch to IgG or IgA was detectable on day 6 by surface staining and IgG and IgA secreting cells were found at later time points. CD4 naïve T cells were inferior to memory cells in providing help and among the latter the most effective were CXCR5+ "follicular helper" T cells. We conclude that BCR stimulation and T cell help are not sufficient to stimulate naïve B cells and that innate stimuli are absolutely required for their survival and differentiation.

35. Cytokine memory and flexibility of human polarized memory T cells

Researchers: Mara Messi, Federica Sallusto

We are interested in understanding the signal requirements for the differentiation of naive T lymphocytes into effector and memory T_H1 and T_H2 cells and the epigenetic mechanisms that maintain the identity of the differentiated cells. Recently, we reported that the differentiation of human $CD4^+$ naïve T cells to effector T_H1 and T_H2 cells *in-vitro* is accompanied by the selective acetylation of the histones associated with *Ifng* and *Il4* promoters respectively, with subsequent high level protein expression. Moreover, we found that circulating memory T_H1 and T_H2 cells (identified by the expression of the chemoattractant receptors CCR5 and CRTh2, respectively) carry acetylated histones at the expressed cytokine gene. In these cells however, the hypoacetylated cytokine gene is not irreversibly silenced and most human memory T_H1 and T_H2 cells, when stimulated under opposite polarizing conditions, acquire the capacity to produce both IFN-γ and IL-4. These results suggest that histone acetylation contributes to imprint and maintain the cytokine memory and indicate that human memory T cells maintain flexibility of cytokine gene expression, a property that is not shared by mouse T cells that become rapidly committed following stimulation.

Publication n. 078

36. Nuclear localization of Th1- and Th2-specific genes in human memory T lymphocytes

Researchers: Mara Messi, Federica Sallusto

When stimulated *in vitro* under polarizing conditions mouse $CD4^+$ T cells become rapidly committed to the Th1 or Th2 lineage. This process is accompanied by repositioning of the silenced cytokine gene to heterochromatic regions. In contrast, most human Th1 and Th2 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 Th2 lineage. These cells did not express the Th1-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 immunoprecipitations demonstrated association of *Tbet* promoter with deacetylated histones in committed Th2 cells but not in naïve and Th1 cells. However, in resting and activated Th2 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. These experiments are done in collaboration with Susannah Hewitt and Matthias Merkenschlager, Imperial College London, London, United Kingdom.

37. Flexible programs of gene expression in human polarized T lymphocytes

Student: Stéphane Chappaz
Supervisor: Federica Sallusto

We have previously shown that human Th2 cells when restimulated under Th1 condition retain the capacity to produce IL-4 while acquiring the capacity to produce IFN-γ. These findings indicate that T cells can maintain memory of the initial polarization while maintaining the flexibility to undergo additional differentiation programs. To investigate whether these characteristics would apply not only to cytokine genes but also to other Th1- or Th2-related genes we performed Affymetrix analysis on Th2 clones that had been recloned under Th2- or Th1-condition. Gene expression was measured in resting and activated T cells. We found that, while IL-4 production was retained in Th2 cells upon Th1-polarization, the expression of other Th2 associated transcripts including several chemokine receptors was lost whereas the Th1-associated chemokine receptors were 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. By comparing three groups of sister clones we also made the unexpected observation that in the activated, but not in the resting state, sister clones express similar pattern of genes, irrespective of their polarization history. This work is done in collaboration with Francesco Bertoni and Andrea Rinaldi, IOSI, Bellinzona, Switzerland.

38. The strength of TCR stimulation regulates survival and effector functions of naïve and memory T cells

Student: Laura Rivino
Researchers: Federica Sallusto, Antonio Lanzavecchia, Jens Geginat
Technicians: David Jarrossay, Isabella Giacchetto-Sasselli

We have previously shown that the strength of TCR stimulation regulates T cell "fitness", i.e. cytokine responsiveness and resistance to death by neglect of naïve T cells. We further analyzed the impact of signal strength on survival capacities, cytokine responsiveness and effector functions of human $CD4^+$ and $CD8^+$ naïve and memory T cells. At low signal strength both naïve and memory T cells proliferated, but expressed low levels of the anti-apoptotic proteins Bcl-2 and Bcl-x_L and died by neglect in the absence of exogenous cytokines. IFN-γ secreting memory cells also showed reduced survival but did not lose IFN-γ producing capacity under these conditions. Naïve T cells proliferated poorly to γc cytokines following weak stimulation, while memory T cells were more responsive to these cytokines and expressed higher levels of IL-15R components. At high signal strength naïve and memory T cells were more resistant to cytokine withdrawal and expressed Ox40, 4.1-BB and CD40, co-stimulatory molecules of the TNFR-family that promote survival of activated T cells and effector-memory transition. Acquisition of receptors implicated in B cell help by $CD4^+$ T cells and up-regulation of perforin by $CD8^+$ T cells were also promoted by a strong signal in both naïve and memory T cells. These results show that following insufficient antigenic stimulation memory T cells have reduced survival capacities and low effector functions, but do not revert to a less differentiated stage.

39. Identification and characterization of IL-10-producing memory T cells

Students: Laura Rivino, Claudia Ruprecht
Researchers: Antonio Lanzavecchia, Federica Sallusto, Jens Geginat
Technicians: David Jarrossay, Isabella Giacchetto-Sasselli

$CD4^+$ memory T cells expressing CCR4 contain cells belonging to the Th2 lineage, CLA^+ skin-homing cells and $CD25^+$ regulatory cells. While both IL-4 and IL-10 were originally believed to be produced by Th2 cells, accumulating evidence suggests that IL-10 producing cells represent a separate lineage that might be involved in immune suppression or B cell help. Following naive T cell priming in the presence of the Th2 polarizing cytokine IL-4 cells acquired a $CCR4^+CCR6^-$ phenotype. Addition of TGF-β a cytokine that inhibits both Th1 and Th2 differentiation, promoted both CCR4 and CCR6 expression. In contrast, IL-10 did not alter the cellular phenotype, but potently boosted IL-10 producing capacities in the absence of costimulation. Consistent with this *in vitro* priming data, *ex vivo* isolated $CCR4^+CCR6^-$ T cells were found to contain virtually all Th2 cells, while $CCR4^+CCR6^+$ cells were non-polarized cells that produced high levels of IL-10. Similar results were obtained when CLA^+, CLA^- and $CD25^+$ T cells were analyzed seperately. Suppression of *in vitro* T cell proliferation and Foxp3 expression were restricted to $CD25^+$ cells and resided in both $CCR6^+$ and $CCR6^-$ fractions. Moreover, both $CCR6^+$ and $CCR6^-$ subsets of $CCR4^+CD25^-$ cells proliferated in response to tetanus toxoid and incorporated BrdU ex vivo, indicating the presence of memory cells with self-renewal capacities. The involvement of $CCR4^+CCR6^+$ memory cells in B cell help is under current investigation.

40. Chemokine receptor expression identifies pre-Th1, pre-Th2 and non-polarized cells among human CD4$^+$ central memory T cells

Students: Laura Rivino, David Jarrossay
Researchers: Mara Messi, Federica Sallusto, Antonio Lanzavecchia, Jens Geginat
Technician: Isabella Giacchetto-Sasselli

Central memory T cells (T_{CM}) express lymph node homing receptors CCR7 and CD62L are largely devoid of effector functions but acquire characteristics of effector memory cells (T_{EM}, i.e. CCR7$^-$ Th1 or Th2) following stimulation with TCR agonists or homeostatic cytokines. Here we show that three chemokine receptors identify functional subsets within the human CD4$^+$ T_{CM} pool. T_{CM} that expressed CXCR3 secreted low amounts of IFN-γ, whereas CCR4$^+$ T_{CM} produced some IL-4, but not IL-5. In response to IL-7 and IL-15 CXCR3$^+$ T_{CM} and CCR4$^+$ T_{CM} invariably generated fully differentiated CCR7$^-$ Th1 and Th2 cells, respectively, suggesting that they represent pre-Th1 and pre-Th2 cells. In contrast, CXCR5$^+$ T_{CM} lacking CXCR3 and CCR4 remained non-polarized and retained CCR7 and CD62L expression upon cytokine-driven expansion. Unlike naïve cells all memory subsets had a low TREC content, spontaneously incorporated BrdU *ex vivo* and contained cells specific for tetanus toxoid. Conversely, responses to Th1-promoting viruses CMV and vaccinia were largely restricted to CXCR3$^+$ T_{CM} and T_{EM}. We conclude that antigen-specific memory T cells are distributed among T_{EM} and different subsets of T_{CM}. These findings also explain how the quality of primary T cell responses could be maintained by T_{CM} in the absence of antigen.

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

Student: Stéphane Chappaz
Researchers: Jens Geginat, Antonio Lanzavecchia, Federica Sallusto

Memory T cells can be divided into follicular helper (T_{FH}) central (T_{CM}) and effector (T_{EM}) 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 preparation 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). This fact limits the extent of detection and requires the introduction of a correction factor. We are now performing extensive sequencing analysis to identify clonotypes in different memory subsets and at different time points. This work is done in collaboration with Cécile Bouneaud, Laurent Ferradini and Christophe Pannetier, INSERM U277, Paris, France and Paolo Dellabona and Giulia Casorati, DIBIT San Raffaele Hospital, Milan, Italy.

42. Foxp3$^+$ human T regulatory cells at sites of human chronic autoimmune inflammatory lesions are identified by expression of CD27

Student: Claudia Ruprecht
Supervisor: Federica Sallusto

There is growing evidence that T regulatory cells, identified by the expression of CD4, CD25 and Foxp3, exert a negative feedback on T cell activation. However, much less is known whether T regulatory cells exert any function in tissues undergoing autoimmune reactions or chronic inflammation. We studied the distribution and function of T regulatory cells in patients with juvenile idiopathic arthritis. We found that $CD4^+$ $CD25^+$ cells were present at increased proportions in synovial fluid as compared to peripheral blood. T cells with high Foxp3 expression and potent *in vitro* suppressor activity were present within a subset of $CD27^+$ cells, whereas $CD27^-$ $CD25^+$ cells were $Foxp3^-$ and devoid of suppressor activity. However, the frequency of regulatory T cells within infiltrating leukocytes did not correlate with disease type (oligoarticular versus polyarticular) or activity. In search of possible "contrasuppressive" mechanisms we found that besides IL-2 other γc cytokines (IL-7 and IL-15) abrogated the suppressive activity of T regulatory cells and that pre-activated T cells become refractory to suppression by T regulatory cells. These results suggest that the low efficiency of cellular interactions and the presence of high levels of IL-15 may hamper regulatory T cell function in inflamed tissues. This work was performed in collaboration with Marco Gattorno and Alberto Martini, "G. Gaslini" Institute and Department of Pediatrics, University of Genoa, Genoa, Italy.

43. Natural antagonists of the chemokine receptor CCR2

Student: Samantha Paoletti
Researcher: Mariagrazia Uguccioni
Technician: Gabriela Danelon-Sargenti

We have reported recently that eotaxin (CCL11), a selective CCR3 agonist, is a natural CCR2 antagonist (publication n. 027). Currently, three selective CCR3 agonists have been characterized: eotaxin, eotaxin-2 (CCL24), and eotaxin-3 (CCL26). We have therefore studied the other selective CCR3 agonists (eotaxin-2 and eotaxin-3) for their ability to bind to the CCR2, in order to assess a potential antagonistic activity. All eotaxins belong to the group of CC chemokines that attract eosinophils, basophils, and TH2 lymphocytes. Owing to this property, these ligands play an essential role in allergic reactions. We have shown that eotaxin-3 also binds to CCR2 on monocytes and CCR2-transfected cells. In contrast to MCP-1 (CCL2), eotaxin-3 does not trigger chemotaxis, intracellular calcium mobilization, phosphorylation of the MAP kinases ERK, or enzyme release through CCR2 in monocytes but inhibits MCP-1-mediated responses, thus acting as a natural antagonist for CCR2. This study also demonstrated that eotaxin-3 has the unique characteristic to promote active movement of monocytes away from the gradient of eotaxin-3 *in-vitro*. This repellent effect is amplified when an additional gradient of MCP-1 is applied showing that the two mechanisms are synergistic. Accordingly, eotaxin-3 induces rapid actin polymerization in monocytes, a prerequisite of migration. Like MCP-1-mediated migration, the repellent

effect is G_i protein dependent as the effect is *Bordetella pertussis* toxin sensitive. This indicates that the involved receptors are G_i protein-coupled like chemokine receptors. Eotaxin-3 was shown to be expressed by vascular endothelial cells and to be essential for endothelial transmigration of eosinophils. Our data provide a mechanism by which two chemokine gradients that are oriented in opposite directions could cooperate in efficiently driving out monocytes from the blood vessel into the tissue. These projects were performed in collaboration with Ian-Clark-Lewis, University of British Columbia, Vancouver, Canada.

Publication n. 084

44. Natural antagonists of the chemokine receptors

Students: Samantha Paoletti, Vibor Petkovic
Supervisors: Mariagrazia Uguccioni, Basil O. Gerber
Technician: Gabriela Danelon-Sargenti

We have extended our ongoing research to identify and characterize in detail, the activity of chemokines that can provide an additional level of control of leukocyte responses. While not characterized extensively yet, the expression profile of eotaxin-3 coincides with a potential role in allergic inflammation. We have provided evidence that eotaxin-3, in addition to its antagonistic activity on CCRR2, acts as a natural antagonist on CCR1 and CCR5 as well. Eotaxin-3 binds to cells transfected with either CCR1 or CCR5 as well as to monocytes expressing both receptors. Further, it inhibits chemotaxis, release of free intracellular calcium, and actin polymerization when cells are stimulated with known agonists of CCR1 and CCR5. An analysis of its three-dimensional structure indicated the presence of two distinct epitopes that may be involved in specific binding to CCR1, CCR2, CCR3, and CCR5. Taken together, our data thus indicate eotaxin-3 to be the first human chemokine that features broadband antagonistic activities, suggesting that it may have a modulatory rather than an inflammatory function. Further, eotaxin-3 may play an unrecognized role in the polarization of cellular recruitment by attracting Th2 lymphocytes as well as eosinophils and basophils *via* CCR3, while concomitantly blocking the recruitment of Th1 lymphocytes and monocytes *via* CCR1, CCR2, and CCR5. The selective CXCR3 agonists MIG, IP-10, and I-TAC attract $CXCR3^+$ cells like $CD45RO^+$ T lymphocytes, B cells, and NK cells. Further, all three chemokines are potent natural antagonists for CCR3, and feature defensin-like, antimicrobial activities. We have shown that I-TAC, in addition to these effects, acts as an antagonist for CCR5. I-TAC inhibited the binding of MIP-1α to cells transfected with CCR5, and to monocytes. Furthermore, cell migration evoked by RANTES and MIP-1β, the selective agonist of CCR5, was inhibited in transfected cells and monocytes, respectively. In two other functional assays, namely the release of free intracellular calcium ($[Ca^{2+}]_i$) and actin polymerization, I-TAC reduced CCR5 activities to minimal levels. Sequence and structure analysis indicate a potential role for K17, K49, and Q51 of I-TAC in CCR5 binding. Our results expand on the potential role of I-TAC as a negative modulator in leukocyte migration and activation, as I-TAC would specifically counteract the responses mediated by many "classical" inflammatory chemokines that act not only *via* CCR3 but *via* CCR5 as well.

Publications n. 108, 109

45. A rich chemokine environment enhances leukocyte migration and activities

Students: Samantha Paoletti, Vibor Petkovic
Researcher: Silvia Sebastiani
Supervisors: Mariagrazia Uguccioni, Basil O. Gerber
Technician: Gabriela Danelon-Sargenti

Leukocyte migration in vitro has been analysed widely, dissecting the different components that are required for this function. However, it remains to be clarified how leukocytes can integrate *in vivo* all the messages, and in particular the ones provided by different chemokines that are concomitantly produced. We now know that certain chemokines can act as natural antagonists, but our present understanding of chemokine-integrated signalling is still at the beginning. We have presented evidence for a novel regulatory mechanism of leukocyte trafficking. In the presence of chemokine complexes composed of agonists and unrelated, non-agonist chemokines cellular responses are strongly enhanced. The increase is synergistic and can be evoked by many but not all chemokines. Chemokine-induced synergism might provide an amplification system in "chemokine-rich" tissues, rendering leukocytes more competent to respond to migratory cues.

46. Prostaglandin E_2 modulates monocyte responsiveness to chemokines

Researchers: Ulf Panzer, Mariagrazia Uguccioni

PGE_2 plays an important role in the immune response by modulating the complex interactions between leukocytes and tissue cells under inflammatory conditions. PGE_2 may possibly influence pro-inflammatory effects of chemokines and chemokine receptors that are among the main regulators of directional leukocyte migration. We analyzed whether PGE_2 affects chemokine receptor expression on human monocytes and their functional responsiveness to inflammatory chemokines. Expression of CCR5 on monocytes is significantly reduced, whereas CCR2 and CXCR4 expression is not affected by PGE_2. However, PGE_2 treatment significantly increases the chemotactic response of monocytes to MCP-1, RANTES and SDF-1. In addition, PGE_2 induces a higher calcium mobilization and actin polymerization upon chemokine stimulation. To better characterize PGE_2 effects, we used specific agonists for the PGE_2 receptors (EP_1 – EP_4) characterized so far. 11-deoxy PGE_1, an EP_2 /EP_4 ligand, could mimic the effects observed using PGE_2. In contrast, the EP_1 agonist, sulprostone, does not modify monocyte responses indicating that the effects of PGE_2 are mediated by EP_2/EP_4 receptors. Monocytes acquire a higher functional responsiveness to MCP-1, RANTES and SDF-1 after exposure to PGE_2, independently of the level of chemokine receptor expression. This mechanism might enhance the local monocyte recruitment under inflammatory conditions, and suggests specific PGE_2 receptor EP_2/EP_4 antagonists as novel agents for the treatment of inflammatory diseases.

47. Chemokine expression in human chronic inflammatory reactions and tumors
Student: Samantha Paoletti
Supervisor: Mariagrazia Uguccioni

We have recently shown that BCA-1 (CXCL13), is expressed by the ectopic follicles that develop in the mucosa of the stomach during Helicobacter Pylori (HP) infection. We have therefore analysed different human autoimmune diseases in order to study the expression in the lymphoid aggregates of BCA-1 and SLC (CCL21), the chemokines that are of crucial importance in the formation and maintenance of the secondary lymphoid structure. Chemokine expression have been analysed by *in situ* hybridization and immunohistochemistry on samples from patients with rheumatoid arthritis and Sjogren's syndrome. All samples showing follicle-like structures express BCA-1 and SLC, indicating a functional role in the formation and maintenance of the extranodal follicles in chronic inflammation. This work is performed in collaboration with Costantino Pitzalis, GKT School of Medicine, London, UK; Antonio Manzo, and Carlo Maurizio Montecucco, University of Pavia, Italy. We have shown previously, that the MALT lymphoma (large B cell lymphoma) that can develop in some of the patients with HP infection is characterized by a massive production of BCA-1. Primary central nervous system lymphoma (PCNSL) is a rare, but often rapidly fatal form of non-Hodgkin B cell lymphoma that arises within the CNS and has a low propensity to metastasize. We have analysed the expression of BCA-1 and SLC on brain biopsy specimens from 24 patients with PCNSL. While BCA-1 was not detected in normal human brain, all brain biopsy specimens containing PCNSL were positive for BCA-1. Double-immunostaining on selected specimens localized BCA-1 to malignant B lymphocytes and vascular endothelium. Tumor cells stained positively for CXCR5, the receptor for BCA-1. In PCNSL, expression of BCA-1 by malignant lymphocytes and vascular endothelium may influence tumor development and/or localization to CNS. This work is done in collaboration with Justine Smith, Casey Eye Institute and Department of Pathology, Oregon Health & Science University, Portland, USA.

Publication n. 077

48. Chemokine expression in lymphoid tissue upon vaccination
Researchers: Silvia Sebastiani, Mariagrazia Uguccioni

We evaluate the expression of chemokines that are produced in the secondary lymphoid organs of rhesus monkeys before and after infection with pathogenic SIV, or upon different kind of vaccination (mucosal vaccines against SIV based on dendritic cells or SIVmac251Δnef). ^{35}S labeled RNA probes have been prepared to detect several chemokines and cytokines in rhesus monkey lymph nodes after vaccination and/or SIVmac251 infection. The distribution and the number of positive cells in normal rhesus monkey lymph nodes after vaccination with SIVmac251Δnef is the same found in normal human secondary lymphoid organs. Following the observation of the pathologist assessing thymus changes during vaccination and challenge, we have analysed the expression of BCA-1 in the thymuses of monkey that presented pathological changes mimicking the alterations observed in humans with myasthenia gravis paralytica and, occasionally in HIV-infected pediatric cases. The samples showing B lymphocytes organized in follicle-like structures at the cortico-medullary junctions where positive for BCA-1, indicating a

possible functional role of this chemokine in the formation and maintenance of the extranodal follicles. This work is done in collaboration with the groups participating to the European Project "Mucosal Vaccines against Human and Simian Immunodeficiency Viruses Based on Dendritic Cells".

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102	**Secondary lymphoid tissue chemokine (CCL21) is upregulated in allergic contact dermatitis** Serra HM, Eberhard Y, Martin AP, Gallino N, Gagliardi J, Baena-Cagnani CE, Ruiz Lascano A, Ortiz S, Mariani AL, Uguccioni M Int. Arch. Allergy Immunol. 2004; 133:64-71	 *1.828*
103	**Contrasting functions of calreticulin and calnexin in glycoprotein folding and ER quality control** Molinari M, Eriksson KK, Calanca V, Galli C, Cresswell P, Michalak M, Helenius A Mol. Cell 2004; 13:125-135	 *16.471*
104	**Central memory and effector memory T cell subsets: functions, generation, and maintenance** Sallusto F, Geginat J, Lanzavecchia A Annu. Rev. Immunol. 2004; 22:745-763	 *54.455*
105	**Development of a human adaptive immune system in cord blood cell transplanted mice** Traggiai E, Chicha L, Mazzucchelli L, Bronz L, Piffaretti JC, Lanzavecchia A, Manz MG Science 2004; 304:104-107	 *28.956*
106	**Depletion of host Langerhans cells before transplantation of donor alloreactive T cells prevents skin graft-versus-host disease** Merad M, Hoffmann P, Ranheim E, Slaymaker S, Manz MG, Lira SA, Charo I, Cook DN, Weissman IL, Strober S, Engleman EG Nat. Med. 2004; Apr 18 (Epub ahead of print)	 *28.740*
107	**Bridging the bone marrow-thymus gap** Manz MG Blood 2004 (in press)	 *9.631*

108 **Eotaxin-3 (CCL26) is a natural antagonist for CCR1 and CCR5**
Petkovic V, Moghini C, Paoletti S, Uguccioni M, Gerber BO
J. Biol. Chem. 2004; Mar 23 (Epub ahead of print) *6.696*

109 **I-TAC/CXCL11 is a natural antagonist for CCR5**
Petkovic V, Moghini C, Paoletti S, Uguccioni M, Gerber BO
J. Leukoc. Biol. 2004 (in press) *4.132*

110 **Efficient production of human monoclonal antibodies neutralizing SARS coronavirus from memory B cells**
Traggiai E, Becker S, Subbarao K, Kolesnikova L, Uematsu Y, Gismondo MR, Murphy BR, Rappuoli R, Lanzavecchia A
Nat. Med. 2004 (in press) *28.740*

111 **Unusual chemokine receptor antagonism involving a map-kinase pathway**
Ogilvie P, Thelen S, Moepps B, Gierschik P, da Silva Campos AC, Baggiolini M, Thelen M
J. Immunol. 2004; 172:6715-6722 *7.145*

112 **Degradation of promoter-bound p65/RelA is essential for the prompt termination of the NF-kB response**
Saccani S, Marazzi I, Beg AA, Natoli G
J. Exp. Med. 2004 (in press) *15.837*

113 **The protein factory**
Molinari M
Protocols of the 30th Seminars of Biological Evolution 2004; 108:117-122 -

114 **Endoplasmic reticulum-associated protein degradation**
Molinari M
Encyclopedia of Biological Chemistry 2004 -

SEMINARS AT THE IRB

2003

January

22 **Marco Baggiolini**, University of Southern Switzerland
"Chemokines: 15 years after IL-8"

24 **Elodie Belnoue**, Department of Immunology, Institut Cochin, Paris
"Leukocyte migration to the brain in experimental cerebral malaria"

February

7 **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"

21 **Steve Pascolo**, Department of Immunology, Institute for Cell Biology, University of Tuebingen, Germany
"Stabilized mRNA as a vaccine vehicle and an adjuvant"

24 **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"

March

12 **Immanuel F. Luescher**, Ludwig Institute for Cancer Research, Epalinges, Switzerland
"Role of CD8 and beta integrins in CTL activation"

13 **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"

27 **Hans Wigzell**, Microbiology and Tumor Biology Center, Karolinska Institutet, Stockholm, Sweden
"Immune protection or enhancement of infection against Chlamydia pneumoniae"

April

1 **Isabelle Maridonneau-Parini**, Institute for Pharmacology and Structural Biology CNRS, Toulouse, France
"Role of tyrosine kinase Hck-positive lysosomes in the formation of podosomes"

2 **Ari Helenius**, Institute of Biochemistry, ETH Hoenggerberg, Zurich, Switzerland
"What viruses teach us about endocytosis"

14 **Martin Bachmann**, Cytos Biotechnology AG, Zurich, Switzerland
"From cross-presentation to cross-priming"

29 **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"

May

6 **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"

8 **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"

12 **Peter Gierschik**, Department of Pharmacology and Toxicology, University of Ulm, Germany
"Regulation of Phospholipase C-beta isozymes by heterotrimeric and Rho GTPases"

15 **Lee-Ann Allen**, Department of Internal Medicine, University of Iowa, USA
"Perturbation of phagocyte function by Helicobacter pylori"

22 **Ernesto Carafoli**, Department of Biological Chemistry, University of Padua, Italy
"The control of cellular Ca2+ signalling: Focus on membrane transporters"

June

6 **Andrew J. Pollard**, Department of Paediatrics, University of Oxford, UK
"Glyconjugate vaccines – how much do we know?"

23 **Silvano Sozzati**, University of Brescia, Italy
"Role of PI3Kg in dendritic cell biology"

July

1 **Thomas Hartung**, European Centre for the Validation of Alternative Methods ECVAM, CCR, Ispra, Italy
"Endotoxic properties of lipoteichoic acids"

8 **Simona Ferrari**, Laboratory of Medical Genetics, University of Bologna, Italy
"Molecular anatomy of the CD40 and AID genes: the Hyper-IgM syndrome"

9 **Giampaolo Merlini**, Department of Biochemistry, University of Pavia, Italy
"Systemic amyloidosis: diagnosis and therapy"

10 **Raffaele Badolato**, Paediatric Clinic, University of Brescia, Italy
"Defects of innate immunity in primary immunodeficiencies"

18 **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"

August

14 **Roberto B. Cattaneo**, Mayo Clinic Rochester, MN, USA
"Measles virus biology: how to make a therapeutic agent from a pathogen"

September

4 **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?"

5 **Beat Imhof**, Department of Pathology, University of Geneva, Switzerland
"The migration process of leukocytes"

5 **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"

25 **Giuseppina Bonizzi**, Department of Pharmacology, School of Medicine, UCSD, La Jolla, CA, USA
"IKK and the control of innate and adaptive immunity"

26 **Antonius Rolink**, Department of Immunology, University of Basel, Switzerland
"Molecular mechanisms guiding early lymphocyte development"

October

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

10 **Alexandra Flemming**, Lymphocyte Interaction Laboratory, Cancer Research UK, London
"SLP-65: an adapter protein functions as a tumor suppressor in pre-B cells"

November

3 **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"

11 **Harald von Boehmer**, Department of Pathology, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA, USA
"Origin and lifestyle of regulatory T cells"

26 **Jagadeesh Bayry**, INSERM U430, Institut des Cordeliers, Paris, France
"Natural antibodies and dendritic cells: maintenance of immune homeostasis"

December

16 **Marco Gattorno**, "G. Gaslini" Scientific Institute, Genoa, Italy
"Synovial enrichment of interferon-α producing cells in juvenile idiopathic arthritis"

INVITATIONS TO CONFERENCES, LECTURES AND SEMINARS

2003

January

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"

February

'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"

March

European School of Oncology Course, 'Biology and treatment of malignant lymphomas', Monte Verità, Ascona, Switzerland
A. Lanzavecchia: "Dendritic cells and malignant lymphomas"
M. Manz: "From stem cells to dendritic 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"

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"

April

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 the Institute 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"

May

Conference on 'Cell therapy: the state of the art and new perspectives', Pavia, Italy
A. Lanzavecchia, invited lecture: "On cellular basis of immunological memory"

Sixth Annual Conference on Vaccine Research, Arlington, VA, USA
A. Lanzavecchia, Keynote Address: "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"

June

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"

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, Palermo, Italy
F. Sallusto: "Migration of dendritic cells and T lymphocytes in the immune response"

July

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"

August

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, Basel, 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"

September

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-C2α: 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"

October *'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"

November *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 Pasteu*r, 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"

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

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 haemato-lymphoid cells', University of Wuerzburg, Wuerzburg, Germany
G. Natoli: "Activation of inflammatory genes by NF-kB recruitment to chromatin targets"

Seminar at the Institute for Immunology, University of Bern, 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 dendric cells and NK cells in lymph node traffic"

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

EVENTS ORGANIZED

11-13.07.2003	Joint meeting UniPathology Zurich, IRB and IOSI Monte Verità Seminar Center, Ascona, Switzerland
18-20.07.2003	EU project Memovax – 'Immunological Memory and Vaccination' Third Informative Meeting Monte Verità Seminar Center, Ascona, Switzerland
26-28.09.2003	IRB PhD Student Retreat Center for Alpine Biology, Piora, Switzerland

AWARDS

14.03.2003	ASIRB/Roche 2002 prize to Nadia L. Bernasconi and Elisabetta Traggiai

NATIONAL AND INTERNATIONAL CO-OPERATIONS

Bernhard Nocht Insitute for Tropical Medicine, Hamburg (Germany)

Cantonal Microbiology Institute, Lugano (Switzerland)

Cellerant, Palo Alto, CA (USA)

Chiron Vaccines, Siena (Italy)

Columbia University, New York, NY (USA)

Cytos Biotechnology AG, Zurich (Switzerland)

DIBIT, San Raffaele Scientific Institute, Milan (Italy)

Emory University, Atlanta, GA (USA)

Glaxo, Smith and Klein, Stevenage (UK)

Guy's Hospital, New Guy's House, Department of Immunology, London (UK)

Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT (USA)

Imperial College, London (UK)

Institut Pasteur, Paris (France)

Institute for Cancer Studies, University of Birmingham, Edgbaston (UK)

Insitute for Rheumatology, GKT School of Medicine, London (UK)

Istituto Cantonale di Patologia, Locarno (Switzerland)

Max-Delbrueck Center for Molecular Medicine, Berlin (Germany)

Millennium Pharmaceuticals, Cambridge, MA (USA)

New York University, New York, NY (USA)

NIH, NIAID, LID, Bethesda, MD (USA)

Novartis Pharma AG, Basel (Switzerland)

Oncology Institute of Southern Switzerland, Bellinzona (Switzerland)

Oregon Health Science University, Portland, OR (USA)

Pfizer Gobal Development, San Diego, CA (USA)

Regeneron Pharmaceuticals, Inc., Tarrytown, NY (USA)

Standford University, Standford, CA (USA)

Swiss Federal Institute of Technology (ETHZ), Zurich (Switzerland)

The Babraham Institute, Cambridge (UK)

University of Alberta, Department of Biochemistry, Alberta (Canada)

University of Basel, Basel (Switzerland)

University of Bern, Bern (Switzerland)

University of Bologna, Bologna (Italy)
University of British Columbia, Vancouver (Canada)
University of Geneva, Faculty of Medicine, Geneva (Switzerland)
University of Insubria, Varese (Italy)
University of Konstanz, Department of Biochemistry, Konstanz (Germany)
University of Marburg, Institute for Virology, Marburg (Germany)
University of Michigan, Medical School, Ann Arbor, MI (USA)
University of Milano-Bicocca, Department of Biotechnology and Bioscience, Milan (Italy)
University of Oulu, Department of Biochemistry, Oulu (Finland)
University of Pavia, Pavia (Italy)
University of Roma "La Sapienza", Roma (Italy)
University of Tokyo, Tokyo (Japan)
University of Tuebingen, Tuebingen (Germany)
University of Ulm, Ulm (Germany)
University of Zurich, Zurich (Switzerland)

AUTHOR INDEX

Numbers from 1 to 48 refer to research reports.
Numbers form *001* to *114* refer to publications.