Institute for Research in Biomedicine

Annual Report 2002

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RESEARCH REPORTS

1. Protein folding and quality control in the endoplasmic reticulum

Researcher: Maurizio Molinari
Technician: Verena Calanca-Piccaluga

Protein quality control in the mammalian endoplasmic reticulum (ER) secures the fidelity of gene expression at the post-translational level. Folded proteins are rapidly transported via ER exit sites to their final destination. Terminally misfolded proteins and orphan subunits of oligomeric complexes are initially retained in the ER, and are then dislocated into the cytosol for proteasome-mediated ER-associated degradation (ERAD). The research in our team is focussed on two aspects of the ER quality control: the mechanisms that allow proper maturation of newly synthesized proteins, and the mechanisms that warrant efficient and rapid destruction of terminally misfolded proteins. N-glycans are added co-translationally to asparagines of proteins expressed in the ER. They facilitate protein folding because they mediate association of newly synthesized polypeptides with the ER-resident molecular chaperones calnexin and calreticulin, thereby exposing folding substrates to ERp57, a glycoprotein-specific oxidoreductase that catalyses formation of disulfide bonds (1). Calnexin and calreticulin knockout mice have been recently generated. Calnexin deficiency causes early post natal death and motor disorders (2); calreticulin deficiency is embryonic lethal (day 13). The aim of this project is to determine consequences of calnexin and/or calreticulin depletion on glycoprotein folding. Relevant questions are: how is folding efficiency affected by the lack of one, the other, or both ER lectins? How tight is the protein quality control in the ER upon depletion of calnexin and/or calreticulin? Can glycoproteins make use of alternative chaperone machineries? Answers to these questions will be given by detailed analysis of the maturation of model glycoproteins in cells derived from calnexin and calreticulin-deficient mouse embryos and in cells in which access to the calnexin/calreticulin cycle is prevented by specific inhibitors. Our model substrates are the spike glycoproteins of Semliki forest virus, of influenza virus and of vesicular stomatitis virus, several mutants of these proteins characterized by temperature-sensitive maturation defects, and cellular substrates such as beta-secretases isoforms involved in Alzheimer's disease, and Pmp22, a protein involved in nerve cells myelination.

Publications n. 051, 061, 064

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

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

Proteins that fail to fold or to assemble correctly in the ER are dislocated into the cytosol for proteasome-mediated degradation. Little is known about the sequence of events occurring between the synthesis in the ER lumen of a protein with folding defects, and its dislocation into the cytosol where proteasome-mediated degradation occurs. Investigation of the fate in the early secretory pathway of a recently identified pancreatic isoform of human beta-secretase (1), a glycoprotein with very inefficient folding, led us to propose a three-steps mechanism for disposal from the ER of aberrant proteins: first, ERAD

candidates are subjected to attempts of folding in the calnexin cycle; second, when they are tagged as terminally misfolded, they are transferred to the BiP/PDI-chaperone system that warrants unfolding of the aberrant structures formed during the first phase; third, the unfolded polypeptide chains are dislocated through the Sec61 channel into the cytosol in a process promoted by the oxidoreductase PDI (2). A number of important questions still remain to be answered: how are proteins to be degraded recognized and tagged as "unable to fold correctly"? What determines extraction of misfolded proteins from the ER-folding cycle and their deviation into the ER-disposal machinery? With the assumption that N-glycans play a fundamental role in the quality control that all glycoproteins expressed in the ER are subjected to, our attention has focussed on the role of ER-resident sugar-binding and sugar-processing proteins in the regulation of the ERAD process. Preliminary results show that the fate of misfolded proteins in the mammalian ER is determined by a fine interplay between the ER-folding sensor UDP-glucose: glycoprotein glucosyl transferase (GT) and EDEM, a recently identified mannose-binding lectin. N-glycan modification by GT maintains unfolded and misfolded proteins in the folding cycle, EDEM selectively extracts misfolded proteins from the folding cycle and deviate them in the ER-disposal machinery.

Publications n. 053, 062

3. The role of redox sensitive proteins in ERAD

Student: Riccardo Vago
Supervisor: Maurizio Molinari

Current models predict an involvement of the ER-resident oxidoreductase PDI in the ERAD process. PDI should make ERAD candidates dislocation-competent by breaking intra- and intermolecular disulfide bonds (reductase activity), and should also assist or trigger dislocation by binding (PDIred) and releasing (PDIox) ERAD candidates in close proximity to the Sec61 translocon complex (redox-driven chaperone activity). The redox-state of PDI is regulated by the oxidoreductins Ero1L-alpha and Ero1L-beta, the latter induced upon unfolded protein response. The aim of this project is to determine if variations in the cellular content of Ero proteins affect degradation of our model substrates. To this end, degradation of several forms of human beta-secretase will be investigated in cells with normal, elevated or reduced level of Ero. Reduction of the intracellular level of Ero will be obtained using "RNA interference".

4. RNA interference to down-regulate expression of ER-resident molecular chaperones and to intervene in protein folding and quality control in the ER

Student: Klara Kristin Eriksson
Supervisor: Maurizio Molinari
Researcher: Paola Lucca

The aim of this project is to determine if it is possible to regulate protein folding and quality control and protein degradation from the ER interfering with the expression of ER-resident chaperones and enzymes involved in these processes. We use RNA-mediated interference (RNAi) to selectively down-regulate expression of several ER-resident molecular chaperones. We obtained a significant down-regulation of calnexin, ERp57,

GT, EDEM, Ero1-alpha and Ero1L-beta in HeLa cells transfected with synthetic 21-nucleotide's duplexes designed to trigger degradation of the target RNAs. The interference normally lasted 3-4 days and reduced the level of the protein-of-interest by 80-90%. Sense and antisense sequences successfully used to design duplexes for transient RNAi were linked by a short loop to promote intracellular annealing and were ligated in an expression vector for mammalian cells (pSUPER) under the control of the RNA polymerase promotor H1. Using this approach, we are trying to generate human and mouse cell lines with persistent RNAi. Work is in progress to determine the phenotype of the cell lines, and to set up a protocol to generate RNAi in cells derived from calnexin and calreticulin knockout mice to determine the consequences of combined deficiency of several chaperones in the same cell line.

5. Recruitment of inflammatory transcription factors to target genes *in vivo*
Researchers: Simona Saccani, Serafino Pantano, Daniela Bosisio, Gioacchino Natoli

Nuclear Factor kappa B (NF-kB) is a family of transcription factors that are rapidly and transiently activated in response to most inflammatory stimuli and are required for transcriptional activation of several inflammatory and immune response genes. We are trying to define the mechanisms regulating recruitment of NF-kB to target genes. We have found 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. We have found that the p38 MAP kinase -that is activated simultaneously to NF-kB- may signal chromatin modifications affecting NF-kB recruitment to a subset of non-accessible genes. We are also trying to understand the molecular determinants of redundancy and specificity in transcriptional regulation by various NF-kB family members. Results obtained to date indicate that most genes can recruit more than one NF-kB dimer, although transcriptional activity of individual dimers at the level of each gene is different.

Publications n. 031, 050

6. Transcriptional repressors in dendritic cell activation and exhaustion
Researchers: Serafino Pantano, Simona Saccani, Federica Sallusto, Antonio Lanzavecchia, Gioacchino Natoli

We have found that a few transcriptional repressors are rapidly down-regulated following dendritic cells (DC) stimulation with bacterial products. We are now analyzing each of these transcriptional repressors individually. The hypothesis we are testing is that rapid down-regulation of transcriptional repressors may be permissive for induction of a subset of rapidly induced genes. We have accumulated evidence indicating the existence of a yet unidentified inflammatory pathway triggering rapid degradation of a repressor and subsequent transcriptional shutdown of its gene. Characterization of this pathway is currently undergoing. The role of transcriptional repressors in triggering and maintaining (at the level of specific genes and *in vivo*) chromatin conformations that are not permissive

for transcription is being evaluated. 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), which is erased upon activation and then subsequently restored, concurrently with post-induction transcriptional repression. These results indicate that H3 Lys9 demethylation and remethylation define a transcriptionally permissive window of time.

Publication n. 063

7. Identification of cis-acting elements regulating IL-12 p40 production in dendritic cells

Researchers: Daniela Bosisio, Simona Saccani, Gioacchino Natoli

With respect to monocytes and macrophages, dendritic cells are endowed with a much higher IL-12 production capacity. The ability to transcribe the IL-12 p40 gene at very high levels is acquired during differentiation from monocytes in vitro. However, the distant regulatory regions controlling IL-12 p40 expression in DCs or in other cells have not been identified to date. In order to identify the putative DC-specific cis-acting regulatory elements responsible for high level IL-12 p40 transcription, we will use two complementary approaches: DNAase hypersensitivity (HS) assays and chromosome walking using Chromatin Immunoprecipitation (ChIP) with antibodies recognizing acetylated histones and sets of primers amplifying regions along the p40 gene as well as 5' and 3' of it. We expect to identify one or more regions acquiring DNAase hyspersensitivity and/or histone hyperacetylation during differentiation of monocytes into DCs. Once these regions are identified, we will try to characterize the transcription factors responsible for their regulation, using bioinformatics, in vitro and in vivo DNA binding assays, and eventually one-hybrid screening for novel DC-specific DNA-binding proteins.

8. Stimulation of chemotaxis by the chemokine receptor CXCR4

Student: Elena Palmesino
Supervisor: Marcus Thelen

A hallmark of chemokines is their capacity to stimulate migration of hematopoetic and tissue cells. Expression of the chemokine receptor CXCR4 was shown to be 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 loose 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). Specific GEF's, which selectively active RhoGTPases, in chemokine receptor stimulated cell migration are not known. As a first step we will investigate chemokine receptor-mediated activation of RhoGTPases during B cell maturation and try to characterize the specific GEF's involved. It is plausible that the migration of maturing B cell is regulated by signal transduction pathways that depend on transient phosphorylation

of cytoskeletal proteins. Analysis of differentially phosphorylated cytoskeletal proteins in migrating and non-migrating B cells could provide further insights on the signal transduction mechanisms. Preliminary data suggest that upon stimulation with CXCL12 the pattern of cytoskeletal phosphoproteins in CXCL12 alters with maturation of B cells.

9. RDC1, a putative chemokine receptor

Student: Simona Infantino
Supervisor: Marcus Thelen
Researcher: Sylvia Thelen

RDC1 was previously reported as receptor for VIP, adrenomedullin and calcitonin gene-related peptide. However, more recent studies suggest that RDC1 is a putative chemokine receptor based on its homology to CXC-chemokine receptors. The RDC1 sequence contains a DRYLA/SV and a NPXXY motif, both highly conserved among chemokine receptors. The gene of RDC1 maps to mouse chromosome 1 and human chromosome 2 where also the genes of two other chemokine receptors, CXCR4 and CXCR2 are localized. RDC1 is highly conserved among species (92 % amino acid similarity between mouse and human RDC1) and similar to CXCR4 scan function as HIV-co-receptor. Like for CXCR4 the mRNA encoding RDC1 has also been detected in non hematopoietic tissue and in tumor cells. Expression was reported to occur in heart, kidney, spleen and astrocytes. So far, no ligand has been identified for the receptor. We have investigated the expression of the RDC1 in human tissues. Preliminary *in situ* hybridization studies of human embryonal tissue suggest that RDC1 is expressed in foetal liver and in epithelium, particularly in intestinal epithelium. In addition, using a PCR-based analysis we found transcripts of RDC1 is in monocytes, basophils and lymphocytes subsets, being most abundant in $CD19^+$ cells. A panel consisting of most known human chemokines will be tested for responsiveness of transfected cells expressing epitope tagged RDC1. Together with a more detailed analysis of RDC1 expression in embryonal tissues and in sorted lymphocytes subsets will help to reveal the function of the receptor and its putative ligand.

10. Chemokine receptor mediated signal transduction

Student: Claudia Da Silva Campos
Researchers: Patricia Ogilvie, Marcus Thelen

Antagonism of chemokines on chemokine receptors constitutes a new regulatory principle in inflammation. Eotaxin (CCL11), an agonist for CC chemokine receptor 3 (CCR3) and an attractant of eosinophils, basophils, and Th2 lymphocytes, was shown to act as antagonist for CCR2, which is widely expressed on leukocytes and is essential for inflammatory responses. We found direct evidence for a novel mechanism of chemokine receptor arrest by endogenous ligands. We could show that binding of eotaxin to CCR2 stimulates the MAP kinases ERK1/2. Activation of the MKK1/2-ERK pathway is indispensable for eotaxin-mediated attenuation of CCR2 function, as inhibition of ERK phosphorylation abolishes the arresting effect. ERK is also activated by CCR2 agonists, e.g. MCP-1 (CCL2). However, the involved pathways are different. By contrast to MCP-1-mediated signaling, eotaxin-induced ERK phosphorylation requires PI□3-kinase activity but is independent of Src kinase activation and of association of CCR2 with membrane lipid rafts. Furthermore, receptor phosphorylation is not induced by eotaxin and is not required

for ERK activation. Thus, eotaxin activates a distinct pathway to prevent proinflammatory responses mediated by CCR2. Pertussis toxin treatment abolishes both eotaxin and MCP-1 induced signals. However, eotaxin only marginally stimulates GTPgS binding suggesting that the main role of the G-protein in eotaxin-stimulated CCR2 activation is the formation of a signaling competent scaffold. In line with this we found that pertussis toxin mediated ADP-ribosylation of the G-protein causes its dissociation from the receptor. By contrast, agonist-stimulated receptor internalization is pertussis toxin insensitive. We will further investigate G-protein dependent and independent chemokine receptor signal transduction.

11. Characterization of BRAK mediated signaling

Researchers: Sylvia Thelen, Marcus Thelen

Breast and kidney-expressed chemokine (BRAK; CXCL14), a CXC chemokine that is highly expressed in skin and lamina propria cells of intestine selectively attracts monocytes. CXCL14 was described to stimulate strong chemotactic activity and Ca^{2+} mobilization in human monocytes. Further was reported that freshly isolated monocytes moderately respond to CXCL14, whereas a strong induction of CXCL14 responsiveness was achieved by treatment of the cell for 2-3 days with cAMP elevating agents. The constitutive expression pattern in different tissues qualifies BRAK as a new member of homeostatic chemokines like CXCL12, CCL19, CCL21 etc. However, the receptor of BRAK is not known. We found that in the presence of elevated intracellular cAMP levels, such as in forskolin treated cells, chemokine receptor-mediated responses become attenuated. In particular, the prolonged signaling of CXCR4 is markedly suppressed under such conditions. The observation that BRAK stimulated chemotaxis and calcium mobilization is upregulated in the presence of elevated cAMP levels is intriguing and suggests that expression of the chemokine receptor, its signaling properties, and its desensitization differs from other chemokine receptors. Thus, characterization of the CXCL14 receptor-mediated signal transduction could reveal novel coupling mechanism for $G_{o/i}$-protein coupled receptors.

12. Cellular functions of the class II HsPI3K-C2

Student: Cristina Gomes Fragoso
Researchers: Svetlana Didichenko, Marcus Thelen

We have shown that in quiescent and proliferating cells the class II HsPI3K-C2 undergoes postranslational modification, which is phosphorylation. Stress-dependent and mitotic phosphorylation of HsPI3K-C2 occurs on the same serine residue within a recognition motif for proline-directed kinases. We could show that mitotic phosphorylation of HsPI3K-C2 can be attributed to cdc2 activity, and that stress-induced phosphorylation occurs via JNK/SAPK. Mitotic phosphorylation of HsPI3K-C2 provides an essential signal for proteosome-dependent degradation of the protein at the M/G1 transition of the cell cycle. We found that over expression of HsPI3K-C2 and defined domains of the protein, which alter the level of the endogens enzyme or change its subcellular localization, affects 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 degradation during mitosis, the data suggest that HsPI3K-C2 is part of a checkpoint control in M phase. Characterization of the mechanisms of HsPI3K-C2-dependent signal

transduction and elucidation of the role of this enzyme in regulation of cell proliferation and its contribution to tumor formation is a major focus of the project.

13. Identification of the contact surfaces between human complement factor 5a and its receptor

Student: Vibor Petkovic
Supervisor: Basil Gerber

G protein coupled receptors (GPCRs) are activated by an amazing variety of agonists, both in terms of size and chemical difference. The binding and activation domain of small ligands such as catecholamines is well defined and lies in the pocket composed of the helical transmembrane segments. Much less is known about large, proteinacous ligands such as complement factor 5a (C5a), which are believed to mainly interact with the extracellular segments of their respective receptors. Previous mutational analyses in a genetic yeast system, together with subsequent pharmacological studies, have identified a number of ligand-interacting residues in the transmembrane helices of the C5a receptor (C5aR), which comprise at least part of the receptor switch, however. In an additional saturation mutagenesis screen in yeast, we have now identified three C-terminal amino acids of C5a that are necessary for receptor activation and that we think to interact with the receptor's transmembrane residues. We are currently testing libraries with all possible amino acid combinations at the crucial positions in C5a and the C5aR against each other to determine, by functional complementation in yeast, which residues exactly mediate the activation of the C5aR by C5a. Successfully identified interactions would then be corroborated further in pharmacological studies. The identification of these intermolecular contacts would constitute, as a "proof of principle", that the activation mode of GPCRs responding to very diverse ligands can be much more conserved than previously assumed.

14. The chemokine CCL22 (MDC) is an agonist for CCR3

Students: Vibor Petkovic, Samantha Paoletti
Researchers: Basil Gerber, Mariagrazia Uguccioni

As described previously, we have initiated our search for natural chemokines which, apart from their known agonistic activities, act as antagonists as well. Quite surprisingly, we have identified novel agonistic activities for some chemokines as well, and are currently focusing on CCL22 (MDC), a chemokine previously thought to act on the chemokine receptor CCR4 exclusively. CCR3-transfected cells respond with a transient increase of intracellular Ca^{2+} to CCL22 stimulation, albeit with low potency and efficiency. CCL22 desensitizes their Ca^{2+} response to CCL11 (eotaxin), the canonical CCR3 agonist. CCL22 induces chemotaxis of these cells at low micromolar concentrations, which can be completely blocked by a CCR3-specific antibody. Potency and efficiency of the response are slightly weaker but comparable to other known CCR3 agonists, such as eotaxin-3 and MCP-2. Sub-stimulatory concentrations of CCL22 do not inhibit CCR3-mediated responses. Taken together, these results indicate that CCL22 is a (weak) full agonist for CCR3 rather than a partial agonist or an antagonist. Currently, we are determining the binding properties of CCL22 for CCR3 and its agonistic effects on eosinophils, the major $CCR3^+$ cell population. CCL22, thought to exclusively act on $CCR4^+$ Th2 cells in chronic

allergies, may thus be capable of simultaneously attracting eosinophils, which are the major effector cells in these conditions.

15. Natural antagonists of the chemokine receptor CCR2

Student: Samantha Paoletti
Researchers: Patricia Ogilvie, Marcus Thelen, Mariagrazia Uguccioni
Technician: Gabriela Danelon-Sargenti

We have reported recently that eotaxin (CCL11), a selective CCR3 agonist, is a natural CCR2 antagonist. Eotaxin and MCP-1 (CCL2) are often co-expressed in pathological conditions, as allergic reactions. MCP-1 is a strong stimulus for enzyme release from monocytes, and histamine secretion from basophils, whereas eotaxin attracts CCR3 positive cells without stimulating release through CCR2. Thus, while eotaxin efficiently recruits leukocytes to sites of injury it tempers at the same time the inflammatory response by attenuating secretion of proinflammatory mediators. Surprisingly, and differently from agonist signalling, eotaxin mediates ERK-2 phosphorylation. This phosphorylation is independent from the activation of receptor-coupled G proteins and Src kinases, from receptor phosphorylation and from the positioning of the receptor in lipid micro domains. 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, like the one showed by Eotaxin, on MCP-1 induced responses. While eotaxin-2 does not bind to CCR2, eotaxin-3 binds to CCR2 with an affinity similar to MCP-1. In contrast to MCP-1, eotaxin-3 does not trigger any response in monocytes but inhibits MCP-1-mediated responses, acting as a natural antagonist for CCR2. In addition to this antagonistic effect and unexpectedly, eotaxin-3 promotes active movement of monocytes if cells are pre-exposed to the chemokine before assessing their migratory capacity *in-vitro*. Our data suggest that eotaxin-3 plays a role in the subtle regulation of monocyte responses in inflammation and might contribute to the precise positioning of monocytes *in-vivo* by providing both antagonism and migratory capacity enhancement. This eotaxin-3 effect on monocytes is now further investigated. This work is performed in collaboration with Ian-Clark-Lewis, University of British Columbia, Vancouver, Canada.

16. Patterns and kinetics of chemokine expression in human inflammatory and allergic disease

Student: Samantha Paoletti
Supervisor: Mariagrazia Uguccioni
Technician: Ilaria Sartore

We have recently shown that the B cell attracting chemokine 1 (BCA-1/CXCL13) is not only expressed in secondary lymphoid organs, but also in 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 of Secondary Lymphoid Tissue Chemokine (SLC/CCL21), the two chemokines that are of crucial importance in the formation and maintenance of the secondary lymphoid structure. Chemokine expression has been

analysed by *in situ* hybridisation 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 extra-nodal 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.

Upon exposure to the stress signals and after capturing antigens, the Langerin$^+$ LCs of the skin up-regulate CCR7, the chemokine receptor for SLC, which plays an important role in their trafficking to the draining lymphoid organs. We have analysed the expression of SLC in skin from patients with allergic contact dermatitis (ACD) at 2, 10 and 48 hours after antigen exposure. The patterns of SLC production by the upper dermal lymphatic vessels in inflamed skin of ACD patients, demonstrates for the first time that the expression of this chemokine is strongly associated also with an inflammatory response. This work is done in collaboration with Hoarcio Serra, Inmunología, Facultad Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

In collaboration with the Istituti Ortopedici Rizzoli (Bologna, Italy), we have recently shown that chondrocytes can produce various chemokines and express several chemokine receptors. In particular, GRO□ is up regulated in osteoarthritis. We have therefore investigated its effects both on isolated human cells and *in vitro* cultured cartilage explants. GRO□ can induce apoptosis in articular chondrocytes and the induction is dependent upon additional signals from the extra cellular matrix. These findings are of relevance in the understanding of osteoarthritis pathogenesis, in view of the availability of GRO□ chemokine in the joint space in the course of rheumatic disease. We are currently analysing the expression of chemokine receptors on chondrocytes and the responses induced by their selective agonists. This work is performed in collaboration with Rosa Maria Borzì, Laboratorio di Immunologia, Istituto Codivilla-Putti, IOR, Bologna, Italy.

Publication n. 071

17. Chemokine expression in tumours

Student: Samantha Paoletti
Supervisor: Mariagrazia Uguccioni
Technician: Ilaria Sartore

We have shown previously, that the MALT lymphoma (large B cell lymphoma) that can develop in some of the patients with *Helicobacter Pylori* infection is characterised by a massive production of B cell attracting chemokine 1 (BCA-1/CXCL13). We have, therefore, analysed the expression of BCA-1 and Secondary Lymphoid Tissue Chemokine (SLC/CCL21) in several B cell derived extra-nodular lymphomas. These tumours develop in extra nodal sites and acquire the capability to produce BCA-1, that in normal conditions is constitutively and exclusively expressed in secondary lymphoid organs by follicular dendritic cells. This work is done in collaboration with Ennio Pedrinis, Istituto di Patologia, Locarno; Emanuele Zucca and Franco Cavalli, IOSI, Ospedale San Giovanni, Bellinzona.

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 metastasise. We have analysed the expression of BCA-1 and SLC on brain biopsy specimens from 24 patients with PCNSL. While BCA-1 is not expressed 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. Tumour cells stained positively for CXCR5, the receptor for BCA-1. In PCNSL, expression of BCA-1 by malignant lymphocytes and vascular endothelium may influence tumour 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

18. Chemokine expression in macaque lymphoid tissue upon vaccination with SIVmac239Δnef, and challenge with SIV mac251

Researchers: Silvia Sebastiani, Mariagrazia Uguccioni
Technician: Ilaria Sartore

This work is performed in collaboration with the groups participating to the European Project "SIV/HIV vaccines – detecting efficacy and explaining inefficacy". 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. ^{35}S labelled RNA probes have been prepared to detect B cell attracting chemokine 1 (BCA-1/CXCL13) and Secondary Lymphoid Tissue Chemokine (SLC/CCL21) mRNA in rhesus monkey lymph nodes after SIVmac239Δnef immunisation and/or SIVmac251 infection. The distribution and the number of positive cells in normal rhesus monkeys lymph nodes after vaccination with SIVmac239Δnef is the same found in normal human secondary lymphoid organs. Unexpectedly, the pathologist assessing thymus changes during vaccination and challenge has found, in some cases, the presence of ectopic and well defined lymphoid aggregates/nodules. The aggregates mimic the lymphoid follicles characteristic of peripheral lymphoid tissues, and the alterations observed in humans with myasthenia gravis paralytica or in HIV-infected paediatric cases. We have therefore analysed the thymus samples in order to investigate BCA-1 expression in the lymphoid aggregates. As for all human chronic inflammatory disease, that we are currently analysing, BCA-1 is also expressed in the well-defined lymphoid aggregates/nodules of the thymus.

19. Characterization of the newly-discovered chemokine, X

Researchers: Dominic van Essen, Klaus Karjalainen

We recently identified a previously unknown member of the CXC-chemokine family, which we named X. The origins of X are currently unclear: although its cDNA was originally found in mouse lymphocytes, the gene for X is absent from the mouse genome so far sequenced. Nevertheless, most monoclonal antibodies raised against X stain mouse spleen, but not lymph node, in both western blots and histological sections. The closest relative of X is the mouse gene for platelet basic protein (PBP), which is also predominantly expressed in the spleen; however, antibodies raised against X are all unreactive against mouse PBP. We are currently attempting to use anti-X monoclonal antibodies to purify the protein from mouse spleen, to determine whether it is X itself, or another closely related molecule. To identify a cellular receptor for X, we have joined X to the constant region of human IgG1. The resulting chimaeric protein (X-g1) binds to mouse

B cells; however most of this activity results from non-specific sticking to surface proteoglycan molecules. We are now using a mutated form of X-g1, which does not bind proteoglycans to screen expression libraries derived from various mouse tissues. *In vivo*, X is located within the red pulp and marginal zones in the spleen, and *in vitro* it is a chemoattractant for T cells, but its role is a mystery. Injection of X-g1 into mice, which may interfere with its normal function, results in an accelleration of antibody production during an immune response. We do not yet know the cellular mechanism for this. Clearly, many aspects of X remain obscure, and we are continuing to piece together whatever clues we find to try to solve the puzzle of its origin and function. This work is done in collaboration with Christiane Ruedl, Cytos Biotechnology AG, Zürich.

20. Molecular analysis of TARPP function

Researchers: Jan Kisielow, Klaus Karjalainen

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

21. Characterization of a novel T cell specific protein

Student: Zuzana Garajova

Researchers: Jan Kisielow, Klaus Karjalainen

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

22. Genomic surprises

Student: Piotr Tetlak
Supervisor: Klaus Karjalainen

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

23. The puzzle of mouse Lag-3 continues

Student: Malgorzata Kisielow
Supervisor: Klaus Karjalainen

Lag-3 is a MHC class II ligand closely related to CD4 surface molecule. Interestingly, it was shown to be expressed on activated T lymphocytes and NK cells. Its potential importance as a negative regulator of T cell activation has been suggested, however its function is still unclear. To gain some understanding of the role of Lag-3 in lymphocyte physiology we have prepared a number of monoclonal antibodies against mouse Lag-3. The antibodies were epitope mapped and tested in surface staining assays. Currently, we are analysing the mode of Lag-3 expression. We also produced and purified truncated Lag-3 molecules in a soluble form for structural and biophysical studies. The truncations contain all four extracellular domains and were prepared in two versions, the glycosylated and de-glycosylated one. Attempts are now being made to crystallize Lag-3 alone or together with class II molecules. In addition, we constructed some CD4/Lag-3 chimeras and want to investigate their effect on T cell activation. Altogether, the above tools will hopefully allow us to look into the nature Lag-3/class II contacts (to map the regions and their affinities to MHC class II molecules), to examine the Lag-3 physiology, and to compare and contrast CD4 and Lag-3 roles in T cell function. Also the precise information about the Lag-3/class II interactions could potentially help to construct the mode of CD4 binding to class II molecules as well.

24. The function of human plasmacytoid dendritic cells

Student: David Jarrossay
Supervisor: Antonio Lanzavecchia

We have previously shown that human plasmacytoid and myeloid dendritic cells (pDC and mDC, respectively) express complementary sets of Toll Like Receptors (TLR) and in response to different microbial stimuli upregulate CCR7, MHC and costimulatory molecules and produce IL-12 or type I IFN. We further analysed the maturation of pDC and mDC in response to various stimuli. We found that after culture with naïve $CD4^+$ or $CD8^+$ T cells, pDC, but not mDC, up-regulated CXCR5, a receptor that drives cell migration into the B cell area where the cognate ligand BCA-1 is produced. Interestingly,

under appropriate stimulatory conditions, pDC also produced BCA-1. These findings suggest that pDC play an important role in B cell responses. We are studying the signals that elict and modulate pDC maturation as well the impact of mature pDC on B cell responses.

25. Regulation of dendritic cell recruitment into lymphatic vessels

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

Migration of maturing dendritic cells (DC) from peripheral tissues to the draining lymph nodes is essential for the induction of T cell responses. Previous work has shown that reverse transmigration from tissues to lymphatic vessels is driven by CCR7, a chemokine receptor expressed on maturing DC, and SLC, a chemokine expressed by lymphatic endothelial cells. We found that the efficiency of DC migration to the lymph nodes is increased by DC themselves, as well as by inflammatory stimuli, through an upregulation of SLC expression in lymphatic endothelial cells. By conditioning the lymphatics we show that higher numbers of DC can be delivered to the draining lymph nodes, leading to a more vigorous T cell response. These results show that recruitment of cells to lymphatic vessels is a highly regulated process and indicate new strategies to increase the efficiency of DC-based cellular therapies.

26. T cell priming by dendritic cells: thresholds for proliferation, differentiation and death and intraclonal functional diversification

Student: Anja Langenkamp

Supervisors: Federica Sallusto, Antonio Lanzavecchia

The variables that influence priming of human naïve $CD4^+$ T cells by dendritic cells (DC) were dissected *in vitro* by analysing the response to the bacterial superantigen TSST or to alloantigens. We show that under conditions that force DC-T cell interactions a single DC can prime up to twenty naïve T cells. Moreover, the strength of antigenic stimulation - as determined by DC numbers, antigen dose, TCR avidity and duration of DC-T cell interactions - drives the progressive differentiation of proliferating T cells from a non-effector $CCR7^+$ stage, to an effector $CCR7^-$ stage and, eventually, to cell death. We also show that the proliferating $CCR7^+$ and $CCR7^-$ populations share clonotypic sequences, demonstrating that the two cell fates can be generated within a single clone. Taken together these results indicate that the strength of antigenic stimulation regulates T cell progression through thresholds of proliferation, differentiation and death. However, the random nature of DC-T cell encounters introduces a critical stochastic element in T cell stimulation which leads to the generation of cells endowed with distinct homing potentials and effector functions within a given T cell clone. This work was done in collaboration with Giulia Casorati and Paolo Dellabona, Cancer Immunotherapy and Gene Therapy Program, H. San Raffaele Scientific Institute, Milano, Italy.

Publication n. 057

27. "Unfit" T lymphocytes generated by insufficient stimulation

Researchers: Jens Geginat, Amanda Gett, Federica Sallusto, Antonio Lanzavecchia
Technicians: David Jarossay, Luana Perlini

T cell stimulation can result in either protective immunity or tolerance. Naïve T lymphocytes primed with immobilized anti-CD3 antibody in the presence of TGF-β or by a short TCR stimulation proliferate in response to IL-2, but are "unfit" since they are programmed to undergo death by neglect and fail to proliferate in response to the homeostatic cytokines IL-7 and IL-15. TCR-stimulated cells that fail to divide are resistant to cell death, but are refractory to both cytokine and TCR stimulation, consistent with an anergic phenotype. In contrast, a prolonged TCR stimulation or CD28 costimulation promotes both clonal expansion and "fitness" of proliferating cells, i. e. resistance to death by neglect and cytokine responsiveness. T cell fitness is associated with a high ratio of protective BCL-2 proteins per mitochondrial mass and enhanced cytokine receptor expression. When transferred into naïve animals, both polyclonal and TCR transgenic unfit T cells disappear rapidly, while fit cells survive, proliferate and accumulate preferentially in the spleen. The state of unfitness induced by insufficient TCR stimulation opens a window of opportunity for tolerance induction.

28. Kinetics of chemokine receptor expression in differentiating $CD4^+$ T cells

Student: Anja Langenkamp
Supervisors: Federica Sallusto, Antonio Lanzavecchia

We investigated the expression kinetics of various chemokine receptors on developing human $CD4^+$ T helper type 1 (Th1) and type 2 (Th2) cells stimulated by myeloid (mDC) or plasmacytoid (pDC) dendritic cells. We found that the Th1-associated chemokine receptors CXCR3 and CXCR6 were upregulated with a faster kinetics compared to the Th2-associated chemokine receptor CRTh2 and CCR3. CXCR3, CCR4 and CCR5 were initially induced both in Th1- and Th2-polarized cultures, indicating promiscuity of chemokine receptor expression in the early phases of T cell activation. pDC and mDC induced distinct patterns of chemokine receptors, with pDC selectively inducing CXCR6. CXCR5, a receptor involved in cell migration to B cell areas, was rapidly and transiently upregulated in naïve, central memory and effector memory T cells, indicating that T cells at different stages of maturation may participate in regulation of B cell responses. CCR7 was also transiently upregulated in all activated T cells indicating that effector T cells can re-acquire the capacity to enter lymph nodes. These data reveal a complex regulation of chemokine receptors in differentiating T cells and suggest that chemokine receptors have differential roles in early and late phases of the immune response by favoring the interaction between antigen-carrying DC and antigen-specific T cells, regulating T-cell dependent antibody production and controlling recruitment of specialized effector T cells to inflamed tissues. This work was done in collaboration with Kinja Nagata, R & D Center, BML, Kawagoe, Japan, and Wu Liu, Millennium Pharmaceuticals, Cambridge, MA.

29. Heterogeneity of central memory T cells

Researchers: Jens Geginat, Federica Sallusto, Antonio Lanzavecchia
Technicians: David Jarossay, Giovanna Bosshard

CCR7 expression on human $CD4^+$ memory T cells distinguishes between non-effector $CCR7^+$ central memory cells and $CCR7^-$ effector memory T cells. We have shown that these subsets have different capacities to proliferate in response to the homeostatic cytokines IL-7 and Il-15 and that some central memory cells differentiate into effector cells upon cytokine-driven proliferation. To further analyze the heterogeneity of human $CD4^+$ memory T cells, we isolated cells expressing various combinations of the chemokine receptors CCR7, CXCR5, CXCR3 and CCR4. The obtained populations had characteristic cytokine producing capacities, proliferation and differentiation potentials in response to antigen or cytokines. $CXCR5^+$ and $CXCR5^-$ central memory cells could be subdivided on the basis of CXCR3 and CCR4 expression: $CXCR3^+$ central memory cells expressed low levels of IFN-γ and differentiated into Th1 effector cells under homeostatic conditions. Reciprocally, $CCR4^+$ cells express low levels of IL-4 and differentiate into Th2 cells. In contrast, cells that are $CXCR3^-CCR4^-$ were unpolarized and differentiated in part to either Th1 or Th2 cells. Thus, chemokine receptor expression in the central memory pool allows the identification of precursors that are programmed for different fates.

Publication n. 047

30. Proliferation and differentiation potential of human $CD8^+$ memory T cell subsets activated by antigen or homeostatic cytokines

Researchers: Jens Geginat, Federica Sallusto, Antonio Lanzavecchia
Technicians: David Jarossay, Giovanna Bosshard

Maintenance of T cell memory is dependent on the homeostatic cytokines IL-7 and IL-15. Human $CD8^+$ naïve and memory T cell subsets were compared for their capacity to proliferate and differentiate in response to antigenic stimulation or IL-7 and IL-15. IL-15 receptor expression and cytokine responsiveness were progressively acquired as a function of CD8 T cell differentiation. In contrast, proliferation and expansion upon TCR stimulation was maximal for naïve and $CCR7^+$ central memory T cells and decreased for the $CCR7^-$ effector memory subsets, due to death of dividing cells. While terminally differentiated $CCR7^-CD45RA^+$ effector memory and naïve T cells had a low *in vivo* turnover, central and effector memory T cells showed high-level spontaneous BrdU incorporation. Following antigenic stimulation all subsets proliferated and differentiated to $CD45RA^-$, $CCR7^-$ effector cells. In contrast, cells expanded with IL-7 and IL-15 maintained their phenotype, with the remarkable exception of central memory $CD8^+$ T cells that generated various types of effector cells including terminally differentiated $CCR7^-$ $CD45RA^+$ cells. Cloning with homeostatic cytokines revealed that central memory cells are functionally heterogeneous, since each cell was committed to a particular differentiation pathway. Indeed, CCR4 expression within the central memory pool discriminated Tc2 cells that produced high levels of IL-4 and CTL precursors that produced IFN and upregulated perforin expression. These results suggest that the heterogeneous pool of $CD8^+$ memory T cells can be maintained by central memory T cells that proliferate and differentiate under homeostatic conditions.

31. Memory and flexibility of cytokine gene expression as separable properties of human Th1 and Th2 lymphocytes

Student: Mara Messi,
Supervisor: Federica Sallusto
Researchers: Antonio Lanzavecchia, Gioacchino Natoli
Technician: Isabella Giacchetto-Sasselli

To gain insights into the mechanisms regulating selective expression and memory of cytokine genes, we investigated histone acetylation status at the *Ifng* and *Il4* promoters in human $CD4^+$ T lymphocytes. Human naïve T cells stimulated under Th1- or Th2-conditions acquired histone hyperacetylation at *Ifng* or *Il4* promoters, which was maintained up to twenty cell divisions. Central memory T cells showed hypoacetylated genes and acquired *Ifng* /*Il4* acetylation and expression upon stimulation. Effector memory T cells showed polarized acetylation patterns *in vivo* but, when stimulated in opposite conditions, acquired acetylation and expression of the alternative cytokine genes. Cytokine flexibility was absent in $CRTh2^+$ memory cells, which failed to upregulate T-bet and to acquire IFN-γ-producing capacity when stimulated in Th1-polarizing conditions. Ectopic T-bet expression conferred $CRTh2^+$ cells the ability to produce IFN-γ. Thus, heritable acetylation at cytokine loci allows fast expression of the corresponding gene while loss of Th1/Th2-specifying transcription factors underlies irreversible commitments. This work was done in collaboration with Kinja Nagata, R & D Center, BML, Kawagoe, Japan.

Publication n. 078

32. Requirements for proliferation and differentiation of human naïve, IgM memory and switch memory B cell subsets

Student: Nadia Bernasconi
Supervisor: Antonio Lanzavecchia

To dissect the requirements for B cell activation, we isolated from human peripheral blood naïve, IgM memory and switch memory B cells and analysed their proliferation and differentiation in response to stimuli delivered through the receptor for antigen (BCR) or through non clonally distributed receptors such as TLRs, CD40 and cytokine receptors. We found that IgM memory B cells selectively proliferated and differentiated in response to CpG oligonucleotides that stimulate via TLR-9. Switch memory B cells were also activated by CpG, but only in the presence of IL-15 or IL-2. Addition of anti-Ig antibody fragments as a trigger for the BCR had only a minor effect indicating that CpG are an optimal trigger for memory B cells. In contrast, naïve B cells showed an absolute requirement for BCR triggering since they proliferated and differentiated only in response to anti-Ig, CpG and cytokines. We also found that switch memory B cells were highly responsive to bystander T cell help delivered by T cells activated by a third party antigen, while IgM memory B cells were less responsive and naïve B cells virtually unresponsive. Furthermore, the response of switch memory B cells was not significantly increased by anti-Ig, indicating that bystander T cell help provides optimal and sufficient trigger. We conclude that, in contrast to naïve B cells, which are dependent on BCR signalling, memory B cells can be selectively activated by polyclonal stimuli such as CpG, cytokines or T cell help in the absence of antigen. Based on these findings we considered the

possibility that a continuous polyclonal activation of memory B lymphocytes may sustain plasma cell generation and antibody production resulting in long term serological memory.

Publication n. 073

33. Long term serological memory maintained by continuous polyclonal activation of human memory B lymphocytes

Student: Elisabetta Traggiai
Supervisor: Antonio Lanzavecchia

Production of antibodies to a specific antigen can be maintained for a lifetime, but the mechanisms that maintain this are still not clear. Current models invoke either long lived plasma cells or persisting antigen. We considered a third possibility, namely that long term memory might be maintained in the absence of the specific antigen through a continuous activation and differentiation of memory B cells in response to polyclonal stimuli derived from microbes or activated T cells. Three lines of evidence point to this conclusion. First, plasma cells, which secrete antibodies to recall antigens are continuously produced *in vivo*, at levels proportional to the frequency of specific memory B cells, even several years after initial antigenic stimulation. Second, under steady state conditions (but not following antigenic boost) there is a strong correlation between the frequency of IgG memory B cells specific for measles virus or tetanus toxoid and the respective serum antibody levels. Third, antigenic stimulation led to a polyclonal activation of memory B cells of irrelevant specificities. Finally, by comparing the kinetics of circulating plasma cells and antibody levels we could estimate the relative contribution of short lived memory and long lived memory plasma cells to the recall response. We conclude that memory B cells play a dual role. In the "antigen-driven mode", they undergo a massive expansion and differentiation to short lived and long lived plasma cells. The latter can sustain serum antibody levels, but do so only for a few months, due to a limited life span of ~50 days. In contrast, in "the polyclonal mode" all memory B cells respond to environmental stimuli by undergoing proliferation and differentiation. In this way, a constant level of plasma cells and derived antibodies could be maintained for a human lifetime.

Publication n. 073

34. The role of Toll like receptors in human B cell activation

Student: Nadia Bernasconi
Researchers: Nobuyuki Onai, Antonio Lanzavecchia

Toll like receptors (TLRs) are triggered by microbial products and initiate transcriptional responses in dendritic cells and other cells of the innate immune system. TLRs are also expressed by B cells, but their role in B cell physiology is less clear. Studies in the mouse system indicate that coengagement of BCR and TLRs trigger antigen specific B cell responses. However TLR4 and TLR9 agonists such as LPS and CpG activate mouse naive B cells even in the absence a BCR trigger. In contrast, in human CpG selectively trigger memory but not naïve B cells, consistent with a role of these receptors in the maintenance of immunological memory. We are therefore characterizing TLR expression in different human B cell subsets. Human B cells express several TLRs, among which TLR9 and TLR10. The latter is not present in the mouse and is expressed only in human B cells but

not in dendritic cells. We have produced TLR10-Ig fusion proteins and used them to generate antibodies to TLR10. In addition, we are producing TLR10 transfectants in CHO cells as a mean to identify TLR10 agonists and signal transduction pathway. Preliminary results indicate that TLRs are highly regulated in naïve and memory B cell subsets.

35. CCR7 reporter and suicide mice

Researchers: Nobuyuki Onai, Klaus Karjalainen, Federica Sallusto, Antonio Lanzavecchia

The chemokine receptor CCR7 is expressed by mature dendritic cells and by naïve and central memory T cells and is required for their localization to the T cell areas. We have introduced in a bacterial artificial chromosome (BAC), the gene encoding for GFP and the monkey difteria toxin receptor under the control of the CCR7 promoter. The large size of the BAC should ensure tissue specific expression of the reporter and suicide gene. The possibility of ablating $CCR7^+$ cells by adding difteria toxin *in vitro* or *in vivo* will be instrumental to test the role of mature dendritic cells and memory T cell subsets.

36. Homeostatic regulation of murine dendritic cell development

Student: Laurie Chicha
Supervisor: Markus G. Manz
Researchers: Nobuyuki 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, DC show a developmental redundancy that is not observed for other cell types of the hematopoietc 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 now 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 currently testing if artificial over-expression of flt3 in flt3-negative progenitors will rescue their DC developmental capacity. This work was done in collaboration with Holger Karsunky and Irving L. Weissman, Stanford University.

37. Human dendritic cell development

Student: Laurie Chicha
Supervisor: Markus G. Manz
Researcher: Antonio Lanzavecchia

In humans as in mice, DCs display different phenotypes, localization, 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 a candidate common lymphoid progenitor (CLPs). We are currently testing which of the restricted progenitors have DC developmental activity *in vitro* and *in vivo*. We found that HSCs, CMPs, and GMPs are capable to generate large numbers of $CD11c^+$ DC in liquid cell culture. Using stroma cell layers and multiple cytokine combinations, we are establishing an *in vitro* system for efficient development of natural interferon producing cells (IPCs, $CD11c^-$ DC) from different progenitors. Also, we are studying *in vivo* human DC development in NOD/SCID and Rag-$2^{-/-}$ gamma common$^{-/-}$ double KO mice. These will enable us to identify human DC lineage origins and to directly compare the earliest genetic events in commitment to either the $CD11c^+$ or $CD11c^-$ DC lineages.

38. Memory T cells for adoptive immunotherapy

Researchers: Markus G. Manz, Alfonso Martín-Fontecha, Amanda Gett, Federica Sallusto, Antonio Lanzavecchia

In most trials that evaluate adoptive T cell therapy in infectious disease and cancer, short lived effector T cells are used. However, sustained immune responses might be dependent on the transfer of T cell memory. We want to test this in an *in vivo* model system by using ovalbumin specific T cells that recognize their cognate antigen on a tumor cell line (OT-I T cells, EG7-OVA T cell lymphoma). So far, we have established conditions to generate memory phenotype ($CCR7^+CD62L^+CD44^+CD69^-$) and effector phenotype ($CCR7^-CD62L^-CD44^+CD69^+$) T cells *in vitro* in an antigen presenting cell free priming system using IL-15 and/or IL-2. As expected, upon *in vivo* transfer memory phenotype CD8 T cells can be found for extended times in secondary lymphoid organs, while effector phenotype CD8 T cells show only short survival and do not home to lymph nodes. We are now testing their *in vivo* functional capacities in mice that are challenged with or carry EG7-OVA tumors.

39. Duration of stimulus and consequences for T cell responses

Researchers: Amanda Gett, Antonio Lanzavecchia

Activation of naïve T cells by antigen presenting cells is a dynamic process during which cell to cell contacts are formed and released. This raises the question of the consequences of varied durations of such interactions for naïve T cell division, differentiation and eventual memory cell formation. Experiments using $CD4^+$ or $CD8^+$ T cells of both mouse and human systems have demonstrated a common requisite of prolonged stimulation for optimal T cell responses. T cell expansion is augmented by increasing the frequencies of cells recruited into division (even when the kinetics are altered by different means of stimulation – whether the variation be in dose or affinity of TCR stimulation, presence or absence of costimulation or the levels of IL-2 available) while proliferation and survival are also enhanced. Similarly, differentiation requires prolonged stimulation with differential cytokine expression patterns (for example loss of IL-2 and gain of IFN-g) being observed as the time is altered. Most importantly, extending duration of stimulation leads to enhanced persistance of activated T cells, corresponding to a progressive efficiency of memory cell generation. Such observations have been confirmed upon adoptive T cell transfers *in vivo*. Accumulation of T cells and preferential migration (compared to lymph nodes) to the blood and most dramatically to the spleen, increases

progressively with longer durations of stimulation. Together the data argue for a critical role of sustained signalling to allow effective $CD4^+$ and $CD8^+$ T cell responses.

40. Cellular and molecular analysis of pre-T cell receptor function

Student: Simona Porcellini
Supervisor: Fabio Grassi

□□ T cell development in the thymus is characterized by differentiation of $CD4^-8^-$ double negative (DN) thymocytes to the $CD4^+8^+$ double positive (DP) stage. Then, differentiation proceeds to either $CD8^+$ or $CD4^+$ single positive (SP) stage depending on the restriction of the □□TCR for class I or class II MHC, respectively. Transition of DN cells to the DP stage is characterized by intense proliferation and is dependent on pre-TCR expression. The pre-TCR is composed by the TCR□ chain (the product of somatic rearrangement of the TCR□ locus) in covalent association with the pre-T□ (pT□) chain (the product of a nonrearranged gene, the expression of which is developmentally regulated). The pre-TCR signals through the CD3 transduction module, i.e. □, □, □chains, and homodimeric □ chains. We previously characterized the property of the pre-TCR to signal independently of an interaction with an exogenous ligand. Transfection of the SCID thymocyte-derived cell line SCIET.27 with a rearranged TCR□ gene leads to expression of the pre-TCR in the plasma membrane. Microscopic analysis of SCIET.27 cells transfected with a TCR□ chain fused to enhance green fluorescent protein (TCR□-EGFP) (SC□□EGFP cells) revealed the accumulation of the green fluorescence in rafts on the cell surface as well as in lysosomes. Constitutive targeting to lysosomes represented a unique feature of the pre-TCR since the □□TCR expressed in the same cell line as well as the □□TCR expressed in two different cell lines did not exhibit this feature. The rapid turnover of the pre-TCR was blocked by sequestering monomeric actin, by expression of a dominant negative dynamin and by inhibition of $p56^{lck}$ activation. Moreover, pre-TCR degradation was blocked by the proteasome inhibitors epoxomicin and lactacystin implicating the cellular ubiquitination machinery in the control of pre-TCR desensitization. We could demonstrate that the adaptor/ubiquitin ligase c-Cbl was phosphorylated and selectively translocated into rafts in pre-TCR but not □□TCR expressing cells. The involvement of the c-Cbl ubiquitin ligase domain in pre-TCR turnover was demonstrated by inhibition of the constitutive degradation of the pre-TCR/CD3 complex through expression of a c-Cbl mutant isoform bearing a dominant negative RING finger ubiquitin ligase domain. Extinction of pre-TCR signaling by endocytosis and degradation may represent an important aspect of pre-TCR function since transgenic pT□ overexpression resulted in increased proliferation of DN cells as well as apoptosis of DP thymocytes and upregulation of pre-TCR expression in a particular genetic context can be tumorigenic.

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017 **Constitutive expression of stromal derived factor- 1 by mucosal epithelia and its role in HIV transmission and propagation**
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019 **Human chondrocytes express functional chemokine receptors and release matrix-degrading enzymes in response to C-X-C and C-C chemokines**
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020 **Macrophages infiltrating the tissue in chronic pancreatitis express the chemokine receptor CCR5**
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023 **Rapid inactivation of stromal cell-derived factor-1 by cathepsin G associated with lymphocytes**
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PUBLICATIONS 2002

No.

050	**p38-dependent marking of inflammatory genes for increased NF-κB recruitment** Saccani S, Pantano S, Natoli G Nat. Immunol. 2002; 3:69-75	***IPF*** *17.500*
051	**Analyzing co-translational protein folding and disulfide-formation by diagonal SDS polycrylamide gel electrophoresis** Molinari M, Helenius A Method Enzymol. 2002; 348:35-42	 *4.068*
052	**Lack of fair play in the T cell response** Lanzavecchia A Nat. Immunol. 2002; 3:9-10	 *17.500*
053	**The disulphide bonds in the catalytic domain of BACE are critical but not essential for APP processing activity** Fischer F, Molinari M, Bodendorf U, Paganetti P J. Neurochem. 2002; 80:1079-1088	 *4.906*
054	**The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells** Engering A, Geijtenbeek TB, van Vliet SJ, Wijers M, van Liempt E, Demaurex N, Lanzavecchia A, Fransen J, Figdor CG, Piguet V, van Kooyk Y J. Immunol. 2002; 168:2118-2126	 *7.145*
055	**Role of molecular chaperones in viral glycoprotein folding** Molinari M, Helenius A In Intracellular and persistent infections, Nobel Symposium 2002; 106, Elsevier, 1-10	 -
056	**Chronic lymphocytic leukemia B cells are endowed with the capacity to attract $CD4^+$, $CD40L^+$ T cells by producing CCL22** Ghia P, Strola G, Granziero L, Guena M, Guida G, Sallusto F, Ruffing N, Montagna L, Piccoli P, Chilosi, M, Calligaris-Cappio F Eur. J. Immunol. 2002; 32:1403-1413	 *5.635*
057	**T cell priming by dendritic cells: thresholds for proliferation, differentiation and death and intraclonal functional diversification** Langenkamp A, Casorati G, Garavaglia C, Dellabona P, Lanzavecchia A, Sallusto F Eur. J. Immunol. 2002; 32:2046-2054	 *5.635*

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060 **Pivotal role of dendritic cell-derived CXCL10 in the retention of T helper cell 1 lymphocytes in the secondary lymph nodes**
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Gagliardi MC, Sallusto F, Marinaro M, Vendetti S, Riccomi A, De Magistris MT
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066 **The instructive role of dendritic cells on T-cell responses**
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067 **Optimizing anti-CD3 affinity for effective T cell targeting against tumor cells**
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072 **Constitutive activation and endocytosis of the complement factor 5a receptor: evidence for multiple activated conformations of a G protein-coupled receptor**
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073 **Maintenance of serological memory by polyclonal activation of human memory B cells**
Bernasconi N, Traggiai E, Lanzavecchia A
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074 **A temporal and spatial summation model for T-cell activation: signal integration and antigen decoding**
Rachmilewitz J, Lanzavecchia A
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075 **Progressive differentiation and selection of the fittest in the immune response**
Lanzavecchia A, Sallusto F
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076 **TCR-independent proliferation and differentiation of human CD4+ T cell subsets induced by cytokines**
Geginat J, Campagnaro S, Sallusto F, Lanzavecchia A
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077 **Expression of B cell attracting chemokine-1 (CXCL13) by malignant lymphocytes and vascular endothelium in primary central nervous system lymphoma**
Smith JR, Braziel RM, Paoletti S, Lipp M, Uguccioni M, Rosenbaum JT
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078 **Memory and flexibility of cytokine gene expression as separable properties of human TH1 and TH2 lymphocytes**
Messi M, Giacchetto I, Nagata K, Lanzavecchia A, Natoli G, Sallusto F
Nat. Immunol., published online Nov. 25, 2002 (2003; 4:78-86) *17.500*

079 **Kinetics and expression patterns of chemokine receptors in human $CD4^+$ T lymphocytes primed by myeloid or plasmacytoid dendritic cells**
Langenkamp A, Nagata K, Murphy K, Wu L, Lanzavecchia A, Sallusto F
Eur. J. Immunol. 2003; 33:474-482 *5.635*

SEMINARS AT THE IRB

2002

January

8 **Matthias Clauss**, Molecular Cell Biology, Max-Planck-Institute for Physiological & Clinical Research, Bad Nauheim, Germany
"New insights into endothelial cell activation"

14 **Luciano Adorini**, Roche Milano Researches, Italy
"Induction of tolerogenic dendritic cells in the treatment of allograft rejection and autoimmune diseases"

22 **Giuliana Cassese**, Deutsches Rheuma Forschungs Zentrum (DRFZ), Berlin, Germany
"Plasma cell survival niches in lymphoid and chronically inflamed tissues"

23 **Alberto Mantovani**, Departement of Immunology and Cell Biology, Mario Negri Institute for Pharmacological Researches, Milan, Italy
"The role of the long Pentraxin in innate immunity"

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

28 **Jeremy Luban**, Departments of Microbiology and Medicine, Columbia University, New York, USA
"Cyclophilin A function in HIV-1 replication and in CD4+ T cells"

29 **Jacob Rachmilewitz**, Goldyne Savad Institute for Gene Therapy, Hadassah University Hospital, Jerusalem
"Temporal summation of early signalling intermediates produced by successively triggered T-cell receptors"

February

5 **Mario Mondelli**, Department of Infectious diseases, University of Pavia, Italy
"Humoral immune response to hepatitis C virus and viral variability"

19 **Roberto Gherzi**, National Institute for Cancer Research, Genova, Italy
"Rapid degradation of unstable transcripts. The Exosome-AUBPs connection"

22 **Matthias Peter**, Swiss Institute for Experimental Cancer Research (ISREC), Lausanne, Switzerland
"MAP kinase dynamics in yeast"

25 **Eli E. Sercarz**, La Jolla Institute for Allergy and Immunology, San Diego, CA, USA
"Driver clones in autoimmunity and their regulation"

March

6 **Andrew Ziemiecki**, Department of Clinical Studies, University of Bern, Switzerland
"Ephs and ephrins in mammary gland biology"

8 **Mathias Hornef**, MTC/Karolinska Institute, Stockholm, Sweden
"LPS recognition in intestinal epithelial cells: balance between necessity and risk"

11 **James Sutton**, University College London, UK
"B cell developments - the role of TGF-Beta RII"

20 **Ueli Aebi**, Biozentrum, M. E. Mueller Institute for Structural Biology, University of Basel, Switzerland
"Task-sharing between actin and actin-binding proteins: who is doing what?"

28 **Roberto B. Cattaneo**, Professor of Biochemistry and Molecular Biology, Mayo Medical School
"Recombinant viruses for cytoreductive therapy"

April

2 **Christoph Moroni**, Institute for Medical Microbiology, University of Basel, Switzerland
"Role of BRF1 in cytokine mRNA turnover"

22 **Brigitta Stockinger**, Division of Molecular Immunology, The National Institute for Medical Research, London, UK
"Survival, homeostasis and competition in naïve and memory T cell pools"

29 **Tim H. Brümmendorf**, Hematology/Oncology, University of Tuebingen, Germany
"Telomere length dynamics in normal hematopoiesis and in disease states associated with increased stem cell turnover"

May

15 **Wilhelm Krek**, Growth Control Program, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
"SCF ubiquitin protein ligases: their roles in cell cycle control and cancer"

24 **Matthias Wymann**, Institute of Biochemistry, Fribourg, Switzerland
"An integrator of chemokine and GPCR signalling: the lipid kinase PI3K'

27 **Massimo Levrero**, La Sapienza University of Rome, Italy
"Modulation of the p53-related p73 function by post-translational modifications"

June

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

4 **Luca Maria Gambardella, Carlo Lepori**, Dalle Molle Institute of Studies on Artificial Intellingence (IDSIA), Manno, Switzerland
"Swarm intelligence"

7 **Bernhard Moser**, Theodor Kocher Institute, University of Bern, Switzerland
"Follicular homing T cells"

10 **Hidde L. Ploegh**, Department of Pathology, Harvard Medical School, Boston, MA, USA
"Antigen presentation in real time"

11 **Ralf Küppers**, Department of Internal Medicine, University of Cologne, Germany
"Aspects of B cell development and lymphoma genesis in the human"

11 **Riccardo Dalla-Favera**, Uris Professor of Pathology and Genetics, Director, Institute for Cancer Genetics, Columbia University, New York, USA
"Molecular pathogenesis of B cell lymphoma"

14 **Fabio Grassi**, Instructor of Pathology, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA, USA
"Self-limited life span of the pre-T cell receptor"

17 **Tobias Junt**, Institute for Experimental Immunology, Department of Pathology, University of Zurich, Switzerland
"Impact of CCR7 and its ligands on antiviral immune responses in vivo"

20 **Ulf Forssmann**, IPF PharmaCeuticals GmbH, Hannover, Germany
"CCL14/HCC-1, a molecule gets active"

July

18 **Francesco Colotta**, R&D Director Molecular Oncology, Dompé Pharmaceuticals, Italy
"Drug discovery applied to GPCRs: repertaxin as a novel inhibitor of IL-8 activity in post-ischemia reperfusion injury"

19 **Francesco Di Virgilio**, University of Ferrara, Italy
"Extracellular nucleotides, P2 receptors and immune regulation"

22 **Gerd Sutter**, Institute for Molecular Virology, Munich, Germany
"Replication-deficient vaccinia virus MVA as candidate vaccine against viral disease and cancer"

30 **Jutta Kollet**, University of Nebraska Medical Center, USA
"IFN-gamma and LPS inducible recruitment of transcription factors to the proximal Interleukin-12 p35 promoter"

August

14 **Hongmin Li**, Wadsworth Center, New York State Department of Health, Albany, NY, USA
"The structural basis of T cell activation by superantigens"

19 **David N. Posnett**, Professor of Medicine, Cornell University, Weill Medical College, New York, USA
"Significance of oligoclonal expansions of antigen specific CD8+ T cells"

19 **Walter E. Laug**, Division of Hematology-Oncology, Children's Hospital Los Angeles, CA, USA
"Effect of av-integrin antagonists on orthotopic and heterotopic brain tumor growth"

21 **Jeffrey V. Ravetch**, Theresa and Eugene Lang Professor, The Rockefeller University, New York, USA
"Inhibitory signaling modulates peripheral tolerance"

23 **Giampietro Corradin**, Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland
"Use of long synthetic poly-peptides for the rapid screening and development of malaria vaccine candidates"

September

2 **Ken Shortman**, The Walter and Eliza Hall Institute Melbourne, Australia
"The influence of microbial stimuli on the development of murine dendritic cell subtypes "

October

1 **Claudio Basilico**, Department of Microbiology, New York University School of Medicine, New York, USA
"Regulation of bone development by FGF signaling"

2 **Scott Mueller**, Department of Microbiology and Immunity, The University of Melbourne, Australia
"Rapid CTL activation following cutaneous HSV-1 infection due to early antigen presentation in the draining lymph nodes"

10 **Philip M. Murphy**, Chief Molecular Signaling Section, Laboratory of Host Defenses, NIAID, NIH, Bethesda, MD, USA
"Potential role for the fractalkine receptor CX3CR1 in human atherosclerosis"

11 **Michael Reth**, University of Freiburg, Germany
"Early events in BCR signaling"

16 **Fabio Re**, Dana-Farber Cancer Institute, Boston, MA, USA
"Toll-like receptor in dendritic cell activation"

23 **Rafal Pacholczyk**, Institute of Molecular and Genetics, Medical College of Augusta, Georgia, USA
"Visualization of the immunological synapse"

November

12 **Emanuela Corsini**, Department of Pharmacological Sciences, University of Milan, Italy
"Impairment in protein kinase C activation in aging"

18 **Lorenzo Leoni**, Department of Medicine, University California San Diego, La Jolla, CA, USA
"The role of cell-to-cell interaction and microenvironment in the pathogenesis of B cell malignancies"

December

11 **Roberto Sitia**, DIBIT, San Raffaele Scientific Institute, Milan, Italy
"Making and maintaining an efficient antibody factory"

19 **Salvatore Valitutti**, INSERM, Institut Claude de Préval, Toulouse Cedex, France
"On the functional role of the T cell/APC immunological synapse"

INVITATIONS TO CONFERENCES, LECTURES AND SEMINARS

2002

February

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

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

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

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

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

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

March

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

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

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

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

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

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

April

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

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

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

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

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

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

'*Immune mechanism and disease*', Grenada, West Indies
A. Lanzavecchia: "On the cellular basis of immunological memory"

'*Immune memory*', *Lunteren,* The Netherlands
A. Lanzavecchia: "Memory stem cells"

'*Cytokines as natural adjuvants: perspectives for vaccine development*', *Istituto Superiore di Sanità*, Rome, Italy
A. Lanzavecchia: "Interactions between dendritic cells and T cells"

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

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

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

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

May

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

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

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

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

American Society for Microbiology ASM 102nd General Meeting, 'Immunobiology and role in infection and immunity', Salt Lake City, Utah, USA
A. Lanzavecchia: "Regulation of T cell immunity by dendritic cells"

XIII International Congress of Histocompatibility and Immunogenetics, 'Adoptive immunity and therapeutic application', Seattle, Washington, USA
A. Lanzavecchia: "T lymphocyte activation by antigen presenting cells"

The Marcus Wallenberg Symposium at Nobel Forum Karolinska Institute, 'Contemporary topics in immunology', Stockholm, Sweden
A. Lanzavecchia: "T cell recognition and activation"

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

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

Universitè Pierre & Marie Curie, Premières Journées des Cordeliers 'The latest advances in immonotherapy', Paris, France
F. Sallusto: "Control of T cell immunity by dendritic cells"

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

June

Euroconference, 'Interactions between innate and adaptive immunity in mammalian defense against bacterial infections', Flesensee, Germany
A. Lanzavecchia: "Dendritic cells responses"

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

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

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

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

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

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

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

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

Merieux Foundation Symposium 'Therapeutic vaccines against HIV and cancers', Veyrier-du-Lac, Switzerland
F. Sallusto: "Exploiting dendritic cells for T cell priming"

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

'Euroconference: Interactions between innate and adaptive immunity', Goehren-Lebbin, Germany
J. Geginat: "DC, cytokines and the generation and maintenance of T cell memory "

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

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

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

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

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

August *XIX International Congress of The Transplantation Society*, Miami, Florida, USA
A. Lanzavecchia: "Lymphocyte Homing"

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

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

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

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

FEBS Intenational Summer School on Immunology, 'The immune system: genes, receptors and regulation', Ionian Village, West Coast of Peloponese, Greece
A. Lanzavecchia: "T cell priming and deletion"
"Maintenance of serological memory"

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

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

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

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

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

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

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

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

European Macrophage and Dendritic Cell Society EMDS, Basel,
A. Lanzavecchia: "The impact of dendritic cells on T cell response"

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

*Seminar at the University of Milan, Department of Pharmacology, Chemotherapy and Medical Toxicology '*E. Trabucchi*'*, Italy
F. Grassi: "Constitutive activation and extinction of the pre-T cell receptor"

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

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

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

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

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

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

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

Advanced Course in Immunology 2002-2003, Institut Pasteur, Paris Cedex, France
F. Grassi: "Development and selection of □□ lymphocytes"

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

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

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

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

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

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

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

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

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

EVENTS ORGANIZED

14-16.03.2002	Swiss Society for Allergology and Immunology, Annual Meeting, Lugano
03-05.04.2002	Joint meeting with Virginia Tech, Riva San Vitale Vaccine technology and immune responses

AWARDS

29.05.2002	*M. Molinari* receives the Award of the "Fondazione per le Malattie Neurodegenerative" (FMN)
16.10.2002	*M. Manz* receives the Swiss Bridge Award 2002
26.10.2002	*M. Molinari* receives the KITI 2002 award from "Kiwanis Club Mendrisiotto"

NATIONAL AND INTERNATIONAL CO-OPERATIONS

Bernhard Nocht Insitute for Tropical Medicine, Hamburg (Germany)

Brigham and Women Hospital and Harvard Medical School, Boston, MA (USA)

Cantonal Microbiology Institute, Lugano (Switzerland)

Centro Svizzero di Calcolo Scientifico, Manno (Switzerland)

Chiron Vaccines, Siena (Italy)

Cytos Biotechnology AG, Zurich (Switzerland)

F. Hoffmann-La Roche Ltd., Basel (Switzerland)

Friedrich Miescher Institute, Basel (Switzerland)

GSF, Munich (Germany)

Guy's Hospital, London (England)

Hospital 12 de Octubre, Madrid (Spain)

ICR, London (United Kingdom)

ICRF, London (United Kingdom)

INRA, Villenove d'Ornon (France)

Institut Pasteur, Paris (France)

Institute for Cancer Research and Treatment, Candiolo (Italy)

Institute for Microbiology, Berlin (Germany)

Insitute for Rheumatology, GKT School of Medicine, London (United Kingdom)

Istituto Cantonale di Patologia, Locarno (Switzerland)

Istituto di Ricerche Farmacologiche Mario Negri, Milano (Italy)

Istituto Oncologico della Svizzera Italiana, Bellinzona (Switzerland)

Istituto Superiore di Sanità, Roma (Italy)

Lower Saxony Institute for Peptide Research, Hannover (Germany)

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

Micromet GmbH, Martinsried (Germany)

Millennium Pharmaceuticals, Cambridge, MA (USA)

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Ospedale San Raffaele Scientific Institute, Milano (Italy)

R&D Center, BML, Kawagoe (Japan)

Royal Perth Hospital, Perth (Australia)

Salvatore Maugeri Foundation, Pavia (Italy)

St. Thomas' Hospital, London (United Kingdom)

Standford University, Standford (USA)

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

UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ (USA)

University College London, London (United Kingdom)

University of Alberta, Alberta (Canada)

University of Basel, Basel (Switzerland)

University of Bern, Bern (Switzerland)

University of Bologna, Bologna (Italy)

University of Brescia, Brescia (Italy)

University of British Columbia, Vancouver (Canada)

University of California San Francisco (UCSF), CA (USA)

University of Firenze, Firenze (Italy)

University of Freiburg, Freiburg (Germany)

University of Oxford, Oxford (United Kingdom)

University of Roma "La Sapienza", Roma (Italy)

University of Saarlandes, Homburg (Germany)

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University of Tuebingen, Tuebingen (Germany)

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AUTHOR INDEX

Numbers from 1 to 40 refer to research reports.
Numbers form *001* to *079* refer to publications.