

**Invited Speaker Abstracts for the Scientific Symposia,  
which were Received after the Printing Deadline**

September 14, 2009

**Scientific Symposium S1.1**

**Pattern Recognition: Receptors and Signals**

**S1.1/D The IL-1 Receptor / Toll-Like Receptor Superfamily: 10 Years of Progress**

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The IL-1R/TLR superfamily was first defined in detail 10 years ago. Since then, there has been remarkable progress in our understanding of both branches of the superfamily. Ligands have been described for most receptors. Within the IL-1R subfamily, notable examples include IL33 for ST2 and IL-1F6 for IL-1Rrp2. The role of TLRs in the sensing of microbial products has led to a renaissance of interest in innate immune mechanisms. For investigators interested in signal transduction, the area has proved very fruitful in terms of the discovery of new signalling pathways and processes. MyD88 is the universal adapter for the superfamily and its central role in inflammation, host defence and even in certain cancers, has been confirmed from studies in knockout mice. We now have a good understanding of the major components activated by TLRs, notably the TIR domain- containing adapters that initiate signalling following recruitment to TIR domains within the TLRs themselves, the IRAK family of protein kinases that are then recruited, and a series of ubiquitination and phosphorylation reactions that ultimately lead to the activation of transcription factors such as NF-kappaB and IRF family members. The structural basis for signalling is still poorly understood however, and we have no appreciation of the kinetics involved in the pathways. Additional components and regulatory cross-talk from multiple signals also continue to be discovered. Genetic variation in signalling components such as in IRAK4, Mal and Unc93b however highlight the importance of these pathways in human health and disease. I will discuss our recent findings of a novel component in TLR4 signaling termed TAG, which specifically inhibits TLR4 signaling from endosomes, and the emerging role of miRNAs as key regulators of TLR signalling events.

**Scientific Symposium S4.1**  
**Intervention**

**S4.1/B Chemokine Reversal of CTLA-4 Induced T-Cell Anergy: Potential for Therapeutic Intervention**

Christopher E. Rudd

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While CTLA-4 is a major regulator of anergy and autoimmunity, the mechanism that reverses T-cell anergy has not been identified. Here, we show that chemokines (SDF-1/CXCL10/Rantes/CCL21) can readily reverse CTLA-4 induced anergy via GPCR reversal of CTLA-4 inhibition of extracellular signal-regulated kinases (ERKs) and microcluster formation. Conversely, CTLA-4 assisted chemokines by releasing T-cells from the TCR 'stop-signal' and enabling direction migration. Chemokines further reversed CTLA-4 limits on T-cell-APC dwell-times as seen with in vitro conjugates and by real-time imaging of T-cell motility and interaction with dendritic cells in lymph nodes. Our findings identify for the first time a receptor-signaling pathway responsible for reversal of CTLA-4 induced T-cell anergy, and introduces a new model of a CTLA-4-chemokine cooperativity in regulating T-cell responses.

**S4.1/D Lymphangiogenesis: Mechanisms and Therapeutics Development**

Kari Alitalo and collaborators

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The lymphatic vascular system is crucial for the regulation of tissue fluid homeostasis, elicitation of immune responses, and fat metabolism. The lymphatic vessels are essential for life, but may become harnessed for sinister purposes in pathological conditions; tumors utilize the lymphatic vessels as routes for metastasis. On the other hand, surgical or other damage to the lymph nodes and the collecting lymphatic vessels may lead to lymphedema, a debilitating condition characterized by chronic tissue edema, impaired immunity, and accumulation of subcutaneous fat. We need to know the molecular and cellular mechanisms regulating the growth and maturation of lymphatic vessels in both health and disease, and especially the pathways showing promise in the inhibition of metastasis and the therapeutic treatment of lymphatic vessel damage and malfunction.

**Scientific Symposium S2.2**  
**Antigen Presentation and Activation**

**S2.2/B NOD2 Function in Antigen Presenting Cells**

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NOD2 is a pathogen recognition receptor expressed in monocyte lineage and intestinal epithelial cells that senses the bacterial cell wall component muramyl dipeptide (MDP). Mutations in the ligand recognition domain of NOD2 are associated with Crohn's disease (CD). These mutations result in defective stimulation of NOD2 following MDP exposure and lead to loss of ability of NOD2 to activate NF- $\kappa$ B, mediate cytokine and defensin release and induce TH17 T cell responses. How these loss of function effects lead to the pro-inflammatory changes observed in CD is unclear. We have investigated NOD2 function in human dendritic cells (DCs), prototypic antigen presenting cells of the immune system, and found activation of NOD2 results in direct induction of autophagy in DCs that affects bacterial handling and antigen presentation. Defects in this pathway are present in CD DCs expressing 1007fsinsC NOD2. We have studied NOD2 function further in terms of cross-talk with TLR2 using large scale gene expression profiling and proteomics approaches and found idiosyncrasies of NOD2 signaling that impinge on several aspects of antigen presentation by DCs after NOD2 triggering. These results will be discussed.

September 15, 2009

**Scientific Symposium S1.4**  
**Innate Lymphocytes**

**S1.4/A Lymphoid Stress-Surveillance by Unconventional T cells**

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The investigation of  $\gamma\delta$  T cells has identified a rapid lymphoid-stress surveillance response to infectious and non-microbial tissue perturbation. In addition to providing local protection, this response provides an immediate source of cytokines, chemokines and other functions that can substantially affect downstream, adaptive immunity. Thus, lymphoid stress-surveillance is likely to underpin aspects of inflammation, tumour immunology, infectious disease, and autoimmunity. Recent experiments from our and our collaborators' laboratories have identified striking molecular mechanisms by which  $\gamma\delta$  cells meet the requirements of stress-surveillance. For example, high response frequencies can reflect developmental focussing of the  $\gamma\delta$  repertoire by thymic selection events driven by novel T cell regulators expressed by epithelial cells. Rapid responses may also be achieved via the primary activation of  $\gamma\delta$  T cells by non-clonotypic receptors, such as NKG2D, that is engaged by "stress-ligands" upregulated on dysregulated epithelial cells. Following ligand recognition, the rapid functional responsiveness of  $\gamma\delta$  cells can be facilitated by the cells' developmental pre-programming. By these various criteria, the unique biology of large numbers of unconventional T cell biology becomes better defined, with both biological and clinical implications.

**Scientific Symposium S2.3**  
**Lymphocyte Signalling**

**S2.3/C Qualitative and Quantitative Analysis of Signal Transduction Pathways in T-Lymphocytes**

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The immune system plays a central role in the maintenance of the body's homeostasis. As it is the case with any other cell of or body, immune cells are regulated by external stimuli which trigger specific receptors that are expressed on the cell surface. Subsequently, the signals are further propagated into the intracellular environment where they initiate multi-component signaling cascades that finally end within the nucleus and (co-) determine (together with other signaling cascades) the immune response. During the past 30 years immunologists have gathered a lot of information regarding the molecular organization of signal transduction networks in T-lymphocytes and, hence, have obtained a quite good understanding of how some TCR-mediated signaling pathways are organized on the molecular level. This knowledge has strongly facilitated the development of new pharmaceutical agents that can be used to manipulate the immune system (e.g. Rapamycin, FK506 and Cyclosporin A and Rituximab). Similarly, agents that interfere with TH1- or TH2-type immune responses are now being used as therapeutic agents in diseases such as rheumatoid arthritis or multiple sclerosis. However, despite the enormous knowledge that has been gained, we are still far away from understanding "the immune cell" as a cellular system. Indeed, while the vertical sequences (from the cell surface to the nucleus) of a variety of signaling cascades are well understood, not very much is known how individual signaling pathways are horizontally interconnected with each other (= how does one signaling pathway influence the other). However, exactly this knowledge is mandatory for a global understanding how immune cells work and, even more importantly, why immune cells sometimes do not work as they should work (e.g. in autoimmune diseases). Moreover, a global understanding of the signaling networks in immune cells is required to predict side effects of novel immune-regulating drugs, to identify new targets for immune intervention and to understand the molecular basis of immune deviation. In close cooperation with mathematicians and theoreticians we have set up a qualitative/binary Boolean model of the signaling network that is organized in T-lymphocytes by the T-cell receptor, the co-receptors CD4/CD8 and the accessory receptor CD28. Currently, the model consists of 96 molecules and 129 reactions. The input layer (TCR, CD4/CD8, CD28) is connected with the output layer (transcription factors, nuclear events) via more than 5.000 paths, many of which are negative regulatory. The individual components of the network were implemented into a computer program, called "CellnetAnalyzer" that allows i) the *in silico* manipulation of each individual component of the network and ii) to immediately obtain the predicted/assumed functional consequences of this manipulation. *In silico* manipulation of the network

revealed unexpected predictions about CD28-mediated signalling which subsequently could be verified by performing the appropriate biological experiment. Collectively, these data show the strong potential that emerges from applying systems biology approaches to specific biological questions. Currently we are extending our Boolean model by implementing additional signaling pathways (Chemokine-receptor mediated signaling, IL2R-mediated signaling, Interferon-signalling). Further, within the EU-consortium SYBILLA (Systems Biology of T cell Activation) we attempt to obtain a quantitative/dynamic Network that describes antigen-driven T cell responses.

### **S2.3/D From Cross-Linking to a Conformational Model of B Cell Antigen Receptor Activation**

Jianying Yang, [Michael Reth](#)

The current model of B cell antigen receptor (BCR) activation suggests that BCRs are monomers on the membrane of resting B cells and that multivalent antigen brings monomers together to allow cross-wise phosphorylation of BCR-associated kinases and signalling to occur. We have previously provided biochemical data showing that the BCR has an oligomeric organization on the surface of resting B lymphocytes (1). We also suggested that it is the opening of a closed BCR oligomer rather than the cross-linking of two BCR monomers that drives B cell activation (2). However, this model was not widely accepted as the detected BCR oligomers were regarded as artefacts of the detergent lysis procedure used in this study. We have now used the bifluorescence complementation assay (BiFC) to demonstrate that the BCR forms oligomers in the membrane of living cells. Furthermore, we have identified a BCR mutant, which is defective in oligomerization. This mutant is hyperactive and not stably expressed on the B cell surface. Based on these studies, we propose that oligomers are autoinhibited forms of the BCR where the ITAM tyrosines are not available for signalling and monomers are the active form of the BCR. During exposure to antigen, the monomers were stabilized by the cross-linking antigen and the oligomers are dissolved, the tails become accessible for phosphorylation and binding by kinases thus trapping them in an active conformation. Data supporting this model will be presented and discussed at the meeting.

1. Schamel, W. and Reth, M. (2000). Monomeric and oligomeric complexes of the B cell antigen receptor. *Immunity* 13, 5-14.
2. Reth, M. (2001). Oligomeric antigen receptors: a new view on signaling for the selection of lymphocytes. *Trends Immunol* 22, 356-360.

**Scientific Symposium S3.3**  
**Autoimmunity**

**S3.3/C Complement, B Cells, Neutrophils, Monocytes and Lupus**

Thereza Imanishi-Kari, Jin-Hwan Han, Anastasia A. Kazimirova and Shauna Hutchinson  
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Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by loss of B cell tolerance and subsequent production of high levels of IgG anti-nuclear autoantibodies. In SLE these antibodies form immunocomplexes (IC) with self-nuclear antigens, which are deposited in multiple organs causing loss of function. However, the cellular and molecular mechanisms underlying these events are not fully understood. We use a novel transgenic mouse model of SLE in order to determine: 1. How do B cells lose tolerance to self-nuclear antigens and produce autoantibodies *in vivo*? 2. What are the roles of granulocytes, Fc $\gamma$  receptors (Fc $\gamma$ R) and type I interferon (IFN-I) in the development of SLE? This is important because patients with SLE have been found to express high levels of IFN-I and show increased expression of genes associated with granulopoiesis. In addition, there are convincing reports that Fc $\gamma$ Rs are necessary for the development of SLE. This mouse model is "564Igi", in which the immunoglobulin genes (Ig) encoding an anti-RNA autoantibody of an autoimmune (SWRxNZB) mouse have been inserted into the Ig locus of wild-type C57BL/6 mice. This mouse model is extremely powerful because it presents many of the characteristics found in patients with SLE, such as the ability to produce anti-RNA antibodies of the Th1 type, such as IgG2a and 2b isotypes. Most importantly, even with a wild-type genetic background they develop glomerulonephritis. With an autoimmune genetic background, such as in complement factor 4 (C4)-deficient mice, there is an increase in autoantibody production and acceleration of disease. In 564Igi mice anti-RNA antibody production is MyD88- and TLR7-dependent and T cell-independent. Bone marrow (BM)-derived developing B cells from 564Igi mice have upregulated IFN-I response genes, toll-like receptor 7 (*Tlr7*), *Tlr8* and activation induced cytidine deaminase (*Aid*) genes as compared to developing B cells from wild-type C57BL/6 mice. As a consequence of this upregulation there is high frequency of IgG anti-RNA antibody secreting cells. In addition, there is an increase in granulocytes (neutrophils) and monocytes in the BM, blood and spleen of 564Igi compared to that of C57BL/6 mice. The increase in the frequency of neutrophils and monocytes is clearly associated with the increased expression of activating Fc $\gamma$  receptor, Fc $\gamma$ RIV. The upregulation of these genes and the increase in granulocytes and monocytes is further increased when the mice are C4-deficient. Surprisingly, we found in 564Igi mice that neutrophils and monocytes are the major IFN-I producers in the BM. These results suggest that developing B cells in the BM are activated by IFN-I, upregulating *Tlr7* and *Tlr8*, which lowers the threshold of B cell activation by self-RNA. As a consequence, *Aid* is upregulated and class switch recombination takes place. We further suggest that the IC of

RNA associated with IgG2a/2b autoantibodies are bound by neutrophils and monocytes, which are the major expressors of Fc $\gamma$ RIV, which has the highest affinity for IgG2a ICs. The engagement of the IC-IgG2a with Fc $\gamma$ RIV activates the granulocytes and induces IFN-I production, driving the whole SLE cycle.

### **S3.3/D Immunopathogenesis and Immunotherapy of Psoriasis**

Frank O. Nestle

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Psoriasis is a chronic inflammatory disease with a significant inherited component. There have been major efforts in recent years to discover novel genes predisposing to psoriasis as well as to dissect disease relevant immunological pathways with the aim to develop targeted treatment approaches. Whole genome studies of psoriasis have changed the way we understand disease susceptibility in psoriasis. A genetic variant in the IL-23R is associated with psoriasis and represents one of the prime examples how genetic variation is potentially associated with functional and therapeutic relevance. In the constant search for effector populations relevant to chronic inflammation unconventional T cells have reached centre stage. A novel proinflammatory human skin homing gamma/delta T cell population shows significant disease association with psoriasis. The presentation will discuss our current understanding of the genetic and immunological basis of psoriasis and will provide insights into future therapeutic targets focusing on tissue resident immune cells and novel cytokine pathways.

### **Scientific Symposium S4.4**

#### **Advances in Treatment of Autoimmune Diseases**

##### **S4.4/A B Cell Depletion as Immunotherapy for Rheumatoid Arthritis**

Paul Emery

The therapy of rheumatoid arthritis has changed dramatically. Diseases previously regarded as untreatable are now seen as largely reversible. A major priority is to see patients before permanent structural damage has occurred. In addition to preventing structural damage there is the concept of preventing irreversible immunological change. Though the requirements for immunologically resetting disease are becoming clearer and again the importance of early disease is confirmed. The approach of a remission-induction regime is now being used in several studies and now preliminary data regarding the prevention and progression of the disease phenotype. Once patients have reached remission the requirements for stopping disease successfully are being addressed. The immunological requirements for successful cessation of biologic therapies are also being explored. In addition to a single cytokine blockade of tumor necrosis factor or IL-6 therapy now includes other approaches with different modes of action including B-cell depletion, post-stimulatory blockade and blocking signalling pathways.

**Scientific Symposium S1.4**  
**Innate Lymphocytes**

**S1.4/E KIR-Mismatched NK Cells in Haploidentical Stem Cell Transplantation**

Alessandro Moretta  
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Clinical data from haploidentical haematopoietic stem cell transplantation (HSCT) revealed that NK cells were responsible for remarkable favourable effects in both adult and paediatric high risk leukemias. NK receptors specific for MHC class I molecules including killer Ig-like receptors (KIR) and CD94/NKG2A play a major role in the anti-leukemia effect (mediating either inhibitory or activating signals). Haploidentical HSCT requires an heavy conditioning regimen for the patient and the use of large numbers of T cell-depleted HSC to be grafted. After transplantation, natural killer cells develop from HSC shortly after engraftment and may include "alloreactive" NK cells that kill leukemic cells and prevent GvHD. Alloreactive NK cells are characterized by the expression of KIR that are not engaged by any of the HLA-class I alleles expressed by the patient. Their generation is dependent on the existence of a KIR/HLA-class I mismatch between donor and recipient. Novel important information on the function and specificity of different KIR has recently been obtained by the analysis of donor-derived alloreactive NK cells in a cohort of paediatric patients given haplo-HSCT to cure acute, high risk leukemias. Donor-derived KIR2DL1<sup>+</sup> NK cells isolated from the recipient displayed the expected capability of selectively killing C1/C1 target cells, including patient leukemia blasts. Differently, KIR2DL2/3<sup>+</sup> NK cells displayed poor alloreactivity against leukemic cells carrying HLA alleles belonging to the C2 specificity. Unexpectedly, this was due to recognition of C2 by KIR2DL2/3, as revealed by receptor blocking experiments and by binding assays of soluble KIR to HLA-C transfectants. Remarkably, however, C2/C2 leukemia blasts were killed by KIR2DL2/3<sup>+</sup> (or by NKG2A<sup>+</sup>) NK cells that co-expressed KIR2DS1. This could be explained by the ability of KIR2DS1 to directly recognize C2 on leukemic cells. A role for the KIR2DS2 activating receptor in leukemic cell lysis could not be established. These results may have important clinical implications for selection of optimal donors in haplo-HSCT.

September 16, 2009

**Scientific Symposium S2.5**  
**Immunological Memory**

**S2.5/D Mining the Human Memory Repertoire**

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Memory T cells, B cells and plasma cells represent a repository of the entire antigenic experience of an individual. By studying the specificity and function of memory cells we can gain insights into the human immune response and isolate T cell clones and monoclonal antibodies. I will briefly describe three methods that can be used to interrogate the repertoires of T cells, B cells and plasma cells and I will then focus on two main aspects. The first deals with the identification of subsets of effector and memory CD4<sup>+</sup> T cells and their role in immune surveillance and protection against different classes of pathogens. The second deals with the heterogeneity of the human antibody response to viruses and with the identification of human monoclonal antibodies with broadly neutralizing activity against influenza viruses.

**Scientific Symposium S3.5**  
**Tumor Immunology**

**S3.5/D Myeloid Derived Suppressor Cells**

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Active suppression of tumor-specific T lymphocytes might limit both immune-surveillance and immunotherapy. Several mouse tumors, either transplantable or spontaneously arising, alter the normal myelopoiesis and cause the expansion of a population of CD11b<sup>+</sup>/Gr-1<sup>+</sup> cells in the blood, lymphoid organs and at the tumor site. These cells were recently defined as myeloid-derived suppressor cells (MDSCs). MDSCs represent a conserved response to inflammatory stimuli and are harnessed by growing tumors to exert different functions, including support for stroma and neovasculature formation. However, in addition to their direct activity on tumor growth, MDSCs have been described as powerful inactivators of tumor-specific CD8<sup>+</sup> T cells. Two major MDSC subsets were recently shown to play an equal role in MDSC-induced immune dysfunctions: monocytic- and granulocytic-like. We isolated three fractions of MDSCs, i.e. CD11b<sup>+</sup>/Gr-1<sup>high</sup>, CD11b<sup>+</sup>/Gr-1<sup>int</sup>, and CD11b<sup>+</sup>/Gr-1<sup>low</sup> populations that were characterized morphologically, phenotypically and functionally in different tumor models. *In vitro* assays showed that Gr-1<sup>int/low</sup> cell subset, mainly comprising monocytes and myeloid precursors, was always capable to suppress CD8<sup>+</sup> T-cell activation, while

CD11b<sup>+</sup>/Gr-1<sup>high</sup> cells, mostly granulocytes, exerted appreciable suppression only in some tumor models and when present in high numbers. The CD11b<sup>+</sup>/Gr-1<sup>int</sup> but not CD11b<sup>+</sup>/Gr-1<sup>high</sup> cells were also immunosuppressive *in vivo* following adoptive transfer. Gene silencing experiments indicated that GM-CSF was necessary to induce preferential expansion of CD11b<sup>+</sup>/Gr-1<sup>int/low</sup> subset in the spleen of tumor-bearing mice and mediate tumor-induced tolerance whereas G-CSF, which preferentially expanded CD11b<sup>+</sup>/Gr-1<sup>high</sup> cells, did not create such immunosuppressive environment. GM-CSF also acted on granulocyte-macrophage progenitors in the bone marrow inducing local expansion of CD11b<sup>+</sup>/Gr-1<sup>low</sup> cells. These data unveiled a hierarchy of immunoregulatory activity among MDSC subsets that is controlled by tumor-released GM-CSF. Moreover, they paved the way for the identification of the interplay between GM-CSF and other tumor-released factor in the control of MDSC expansion and activation, allowing us to identify a transcription factors essential for emergency myelopoiesis in tumor-bearing host as a key regulator of MDSC immunosuppressive programme.

### **S3.5/E No Escape**

Gerald Willimsky, Thomas Blankenstein

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It has rarely been questioned that spontaneously occurring cancer cells have to escape T cell attack, even though it has not been directly demonstrated. Recently, it was shown that sporadic immunogenic cancer at the time of initial recognition induces an aberrant rather than a protective T cell response, resulting in tolerance at the premalignant stage, long before tumors become apparent. Thus, in a clinically relevant model cancer cells do not need to escape. General immune suppression is a late event, probably involves immature myeloid cells, requires immunogenic tumors and appears to be a symptom, not the cause of tumor growth. Tumor infiltrating lymphocytes reflect cancer-induced inflammation, rather than immunosurveillance.

### **Scientific Symposium S4.5** **Transplantation**

#### **S4.5/C Fate of Immature Hematopoietic Stem Cells During Embryogenesis**

Aurelie Kieusseian, Isabelle Godin\*, Ana Cumano

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Definitive HSC are first found in the dorsal aorta of the AGM after 35 somites. However the developmental steps that give rise to HSC are unclear. By using an LTR assay into Rag2 $\gamma$ c<sup>-/-</sup> and Rag<sup>-/-</sup> mice, we identified a new population of HSC called here immature HSC that are first detected in the P-Sp at 9dpc corresponding to 15-20 somites. Importantly, when cultured in vitro with TPO and the OP9 stromal cell line, immature HSC evolve into a population of functional HSC capable to reconstitute Rag2<sup>-/-</sup> mice. This result raises the possibility that immature HSC can give rise to conventional HSC. Until now it was accepted that HSC generated in the AGM colonize the fetal liver around 11 dpc where they expand. We demonstrate here that immature HSC precede mature HSC in the FL. A 4-day organ culture of fetal liver isolated between 30-32 somite stage resulted in the expansion and maturation of the hematopoietic progenitors into an adult phenotype and the capacity to reconstitute NK<sup>+</sup> mice. This capacity is accompanied by the up-regulation of MHC class I expression and the down regulation of Rae1, a NKG2D ligand. We show that the presence of mature HSC in FL is at least partially due to their production in situ starting from the immature HSC present in the FL at 30 somites.