Established in 1871

Formerly: Schweizerische Medizinische Wochenschrift

SMW

Swiss Medical Weekly

Supplementum 170

ad Swiss Med Wkly
2009;139(9–10)
March 7, 2009

The European Journal of Medical Sciences

Joint annual congress of the Swiss Society for Allergology and Immunology Swiss Society for Infectious Diseases

Geneva (Switzerland), March 19/20, 2009

Official journal of the Swiss Society of Infectious Diseases,
the Swiss Society of Internal Medicine and the Swiss Respiratory Society

Supported by the FMH (Swiss Medical Association) and by Schwabe AG,
the long-established scientific publishing house founded in 1488

EMH FMH Schwabe
Editores Medicorum Helveticorum

Suppl. 170
ad Swiss Med Wkly
2009;139(9–10)
March 7, 2009

Abstracted / indexed in
Index Medicus / MEDLINE
Web of science
Current Contents
Science Citation Index
EMBASE

Guidelines for authors
The Guidelines for authors are published on our website http://www.smw.ch
Please send all manuscripts in electronic form by e-mail to:
– for manuscripts: submission@smw.ch
– for letters to the editor: letters@smw.ch

All communications to:
EMH Swiss Medical Publishers Ltd.
Swiss Medical Weekly
Farnsburgerstrassse 8
CH-4132 Muttenz, Switzerland
Phone +41 61 467 85 55
Fax +41 61 467 85 56
advertising@emh.ch
marketing@emh.ch
subscriptions@emh.ch

ISSN printed version: 1424-7860
ISSN online version: 1424–3997

Managing editor
Natalie Marty, MD (nmarty@smw.ch)

Papers administrator
Gisela Wagner (gwagner@smw.ch)

Language editors
Thomas Brink, MD; Judith Lutz-Burns, MD; Roy Turnill, MA

Regular subscription price for 2009: CHF 150.– (shipping not included)

Published fortnightly

EMH FMH Schwabe

EMH Editores Medicorum Helveticorum, CH-4010 Basel
Schweizerischer Ärzteverlag AG, Editions médicales suisses SA,
Edizioni mediche svizzere SA, Swiss Medical Publishers Ltd.

OC1

Implications of endogenous retroviruses in murine systemic lupus erythematosus

Baudino L., Santiago-Raber M.-L., Yoshinobu K., Evans L.H., Izui S.
CMU, Pathologie et Immunologie, Genève, Switzerland

Endogenous retroviruses are implicated in the pathogenesis of systemic lupus erythematosus (SLE), as relatively large amounts of the envelope glycoprotein, gp70, were found in serum and in glomerular immune deposits of lupus-prone mice. Since four different classes of endogenous retroviruses, i.e. ecotropic, xenotropic, polytropic (PT) or modified polytropic (mPT), are expressed in mice, we investigated the possibility that a particular class of endogenous retroviruses is associated with the development of murine SLE. We observed more than 15-fold increased expression of mPT env (envelope) RNA in livers of all four lupus-prone mice, as compared with those of nine non-autoimmune strains of mice. This was not the case for the three other classes of retroviruses. Furthermore, we found that many strains of mice expressed defective mPT env transcripts, in addition to intact mPT transcripts, while lupus-prone mice selectively expressed abundant levels of intact mPT env transcripts, but only low or non-detectable levels of the mutant env transcripts. The Sgp3 (serum gp70 production 3) locus on mid chromosome 13 derived from lupus-prone mice was responsible for the selective up-regulation of the intact mPT env RNA, and also promoted the development of autoimmune responses against serum gp70. Finally, we observed that single-stranded RNA-specific TLR7 played a critical role in the production of anti-gp70 autoantibodies. Our data suggest that lupus-prone mice may possess a unique genetic mechanism responsible for the expression of mPT retroviruses, which could act as a triggering factor through activating TLR7 for the development of autoimmune responses in mice predisposed to SLE.

OC2

Live imaging of α4-integrin mediated trafficking of dendritic cells into the CNS during experimental autoimmune encephalomyelitis

Coisne C., Jain P., Enzmann G., Engelhardt B.
University of Bern, Theodor Kocher Institute, Bern, Switzerland

During multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE), autoreactive T cells invading the central nervous system (CNS) must recognize myelin-derived antigens presented by antigen-presenting cells in order to display effector functions. In this process, the role of dendritic cells (DCs) has emerged but the mechanisms regulating their trafficking into the CNS still need to be characterized. We investigated in vivo live imaging of DC recruitment across the spinal cord white matter microvasculature during EAE by intravital fluorescence videomicroscopy. Immature bone marrow-derived DCs were efficiently recruited into the inflamed spinal cord white matter. However, upon LPS-activation, DC recruitment was dramatically impaired. Immature and LPS-activated DCs also demonstrated differences in their ability to subsequently diapedese through the inflamed microvessel wall and transmigrate into the CNS parenchyma. Blocking α4-integrins did not significantly reduce neither rolling nor capture of immature and LPS-activated DCs to the BBB endothelium but did quite abolish their firm adhesion to the microvasculature, preventing their subsequent transmigration within the CNS parenchyma compared to control. This study supports the notion that during EAE DCs may migrate into the CNS, where they display a major role in the perpetuation of autoimmune responses. Therapeutic strategy aiming at blocking α4-integrins with natalizumab in MS, may directly affect DC trafficking into the CNS and thus impair the stimulation and maintenance of the autoreactive immune response within the CNS during the course of the disease.

OC3

Antigen presentation by plasmacytoid dendritic cells contributes to tolerance in experimental autoimmune encephalomyelitis

Hugues S., Kuepfer N., Irla M., Benkhoucha M., Lalive P., Suter T., Fontana A., Reith W.
University of Geneva, Faculty of Medicine, Geneva, Switzerland

In contrast to conventional DCs (cDC), which function as sentinels of the adaptive immune system and initiate T cell immunity and peripheral T cell tolerance, plasmacytoid DCs (pDC) were initially believed to be involved primarily in innate immune responses, particularly via the secretion of type I interferon and other cytokines in response to viral infections. However, like cDC, pDC express MHC class II (MHCII) molecules and recent evidence has suggested that they are also likely to be implicated in adaptive immune responses, particularly in the induction of T cell tolerance. To study the contribution of MHCII-mediated Ag presentation by pDC we have studied the development of Experimental autoimmune encephalomyelitis (EAE) in mice characterized by the selective loss of MHCII expression on pDC. These mice therefore present a unique tool for distinguishing between the roles of pDC in innate and adaptive immune responses. EAE is mediated by encephalitogenic $CD4^+$ T cells that are first primed in secondary lymphoid tissues by the recognition of specific myelin antigens (Ags) presented in the context of MHCII molecules at the surface of specialized antigen presenting cells (APC). The primed T cells then migrate to the central nervous system (CNS), where they are reactivated by a second encounter with their cognate Ag-MHCII complexes displayed by local APC, leading to the effector phase of the disease. It has become clear that cDC function as key APC during both the priming and effector phases of EAE. Our results have suggested that MHCII-mediated Ag presentation by pDC, in contrast, attenuates the severity of EAE, and that this protective role may be mediated by the induction of regulatory T cells (Treg) capable of inhibiting EAE development.

OC4

Regulatory T cells control CNS-infiltration of autoreactive T cells during viral infection without affecting the antiviral immune response

Firner S., Cervantes-Barragan L., Bechmann I., Thiel V., Ludewig B.
Laborforschungsabteilung, Research Department, St. Gallen, Switzerland

Regulatory T cells are essential for suppressing immune responses to autoantigens and therefore help to prevent autoimmunity. During viral infections in the CNS, an indiscriminate regulation of T cells can prevent autoimmune diseases but could also impair the control of viral replication. We analyzed here the impact of regulatory T cells in the control of T cell infiltration to the CNS, virus-induced CNS pathology, and viral clearance using the mouse hepatitis virus (MHV) A59 intranasal infection model; a virus infection that leads to encephalitis and demyelination. MHV infection of "depletion of regulatory T cell" (DEREG) mice, where regulatory T cells can be transiently depleted by diphtheria toxin injection, revealed that the lack of regulatory T cells during MHV infection leads to an increased T cell infiltration and pathology in the CNS. However, antiviral T cells response were not affected by the depletion of FoxP3+CD4+ T cells indicating that regulatory T cells control infiltration of T cells to the CNS without impairing or delaying the antiviral immune response. Moreover, in MHV infected mice, adoptively transferred-myelin oligodendrocyte glycoprotein (MOG35-50)-specific CD4+ T cells proliferated in cervical lymph nodes and migrated to the CNS, suggesting that MHV infection in the CNS induces the activation of self-reactive T cells which are controlled by regulatory T cells to reduce the risk of developing inflammatory CNS disease.

OC5

The transcription factors RUNX1 and RUNX3 are essential for the induction of FOXP3 and the suppression capacity of T regulatory cells

Klunker S., Chong M., Mantel P.-Y., Rückert B., Bassin C., Ziegler M., Meiler F., Akdis M., Littman D., Akdis C.
Swiss Institute of Allergy and Asthma Research, Immunology, Davos-Platz, Switzerland

Inducible CD4+ regulatory T (Treg) cells that are characterized by the expression of the transcription factor forkhead box P3 (FOXP3) and CD25 play an important role in immune homeostasis. Here, we show that stimulation of human CD4+ T cells with anti-CD2-, anti-CD3-, anti-CD28-mAb and the transforming growth factor-β (TGFβ) induce the expression of the Runt-related transcription factor (RUNX) 1 and RUNX3. This induction seems to be a prerequisite for the binding of RUNX1 and RUNX3 to the FOXP3 promoter. We identified three putative RUNX binding sites in the FOXP3 promoter and the functionality of these binding sites was verified in a biotinylated oligonucleotide precipitation assay. We investigated the effect of RUNX1 and RUNX3 binding on the expression of FOXP3 in primary human CD4+ T cells. Mutation of all three binding sites decreased the luciferase reporter gene expression significantly. In addition, combined knockdown of RUNX1 and RUNX3 in human naïve CD4+ T cells diminished their ability to produce FOXP3 after anti-CD2-, anti-CD3-, anti-CD28-mAb and TGFβ stimulation. Knockdown of the DNA-binding RUNX cofactor, core-binding factor-β, in mice resulted in a reduced induction of Foxp3 by TGFβ. Treg cells generated in core binding factor β-deficient mice showed a diminished suppressive function in vitro. RUNX activity and its binding to FOXP3 promoter in inducible Treg cells were essential for full FOXP3-mediated suppression. These data demonstrate a molecular mechanism in TGFβ-induced Foxp3 expression during the generation of functional Treg cells.

OC6

TLR7-dependent accelerated development of systemic lupus erythematosus in TLR9-deficient lupus-prone mice

Santiago-Raber M.-L., Dunand-Sauthier I., Borel P., Uematsu S., Wu T., Mohan C., Akira S., Izui S.
CMU, Pathology and Immunology, Geneva, Switzerland

SLE is a systemic autoimmune disorder characterized by the formation of various autoantibodies and subsequent development of immune complex glomerulonephritis. The pathogenesis of SLE is a complex process, in which MHC-linked and multiple non-MHC-linked genetic factors contribute to the overall susceptibility of the disease. More recently, the possible roles of TLR7 and TLR9 in the development of anti-nuclear autoantibodies, because of their respective recognition of RNA and DNA, has been suggested. To better define the respective contributions of TLR7 and TLR9 to the development of SLE, we introduced the TLR7 or/and TLR9 null mutation into C57BL/6 mice congenic for Nba2 (NZB autoimmunity 2) locus (B6.Nba2) and followed the development of SLE (autoantibody production and mortality due to glomerulonephritis). B6.Nba2.TLR9–/– female mice displayed a markedly accelerated development of SLE (54% mortality at 9 months of age vs. none at 14 months in B6.Nba2 females). This acceleration was associated with an increased production of autoantibodies against nuclear antigens (DNA, histones and ribonucleoproteins, but not chromatin), serum retroviral gp70 and glomerular matrix antigens (collagens and elastin). Strikingly, the expression of TLR7 was up-regulated in B cells and plasmacytoid dendritic cells in these mice. In a marked contrast, the development of SLE in B6.Nba2.TLR9–/– females was completely prevented by the presence of TLR7 null mutation. No mortality due to GN was observed by 14 months of age, and serum levels of autoantibodies including anti-chromatin autoantibodies were comparable or even lower than those of B6.Nba2 female mice. Our results indicate that TLR7 played a critical role in a wide variety of autoimmune responses against nuclear, retroviral and glomerular matrix antigens, while TLR9 was only involved in the development of anti-chromatin autoantibodies, and that the accelerated development of SLE in TLR9-deficient mice was due to an enhanced TLR7-dependant B cell and plasmacytoid dendritic cell activation.

OC7

Bispecific designed ankyrin repeat protein as human high-affinity IgE receptor antagonist

Eggel A., Amstutz P., Stadler B.-D., Vogel M.
Universitätsinstitut für Immunologie, DOLS, Bern, Switzerland

Bispecific receptor antagonists might be significantly more efficient than monospecific inhibitors. Especially, if more than one receptor-ligand binding-site is involved in the interaction. Simultaneous targeting of different epitopes on a receptor enlarges the binding surface and thus increases affinity and potency of an antagonist. In order to test this hypothesis IgE and its high-affinity receptor (FcεRI) were chosen as a model. The interaction of IgE with the α chain of FcεRI (FcεRIα) occurs via two distinct binding-sites. We aimed to generate a bispecific FcεRIα antagonist that recognizes two epitopes interfering with the IgE binding-sites. Recently described DARPins (designed ankyrin repeat proteins) were used for such purpose. They represent a novel non-immunoglobulin like binding scaffold. DARPins were generated from a consensus sequence of natural ankyrin proteins occurring intra- and extracellularly in all organisms. As the name suggests, ankyrin repeat proteins evolved to anchor proteins to each other. The modular architecture of DARPins and the defined randomized interaction residues in the consensus sequence allowed to generate DARPin libraries with high diversities. We selected binders against the extracellular part of FcεRIα from such DARPin libraries using ribosome display. Different anti-FcεRIα DARPins competing IgE binding were rendered bispecific and bivalent using a standard $[gly_4\text{-}ser]_4$ protein linker. The ability of these different antagonists to inhibit allergen induced basophil degranulation was assessed in functional cell assays. One bispecific DARPin was highly effective. It showed a synergistic inhibitory effect in respect to simultaneously applied monospecific DARPins. Hence, we demonstrated the feasibility to produce bispecific receptor antagonists with enhanced inhibitory potential. Moreover, we showed that *in vivo* application of FcεRIα-antagonists might bear high risks. Cross-linking of receptor bound antagonists by an antibody simulated allergen induced receptor aggregation and resulted in the release of pro-inflammatory mediators.

OC8

Human basophils and eosinophils are the direct target leukocytes of the novel IL-1-family member IL-33

Pecaric Petkovic T., Didichenko S., Spiegl N., Kämpfer S., Dahinden C.A.
Institute of Immunology, University of Bern, Institute of Immunology, Bern, Switzerland

In mice, IL-18 regulates Th1- or Th2-type immune responses depending on the cytokine environment and effector cells involved, and the ST2-ligand, IL-33, primarily promotes an allergic phenotype. Human basophils, major players in allergic inflammation, constitutively express IL-18 receptors, while ST2 surface expression is inducible by IL-3. Unexpectedly, freshly isolated basophils are strongly activated by IL-33, but, in contrast to mouse basophils, do not respond to IL-18. IL-33 promotes IL-4, IL-13 and IL-8 secretion in synergy with IL-3 and/or FcεRI-activation, and enhances FcεRI-induced mediator release. These effects are similar to that of IL-3, but the signaling pathways engaged are distinct since IL-33 strongly activates NF-kB and shows a preference for p38 MAP-kinase, while IL-3 acts through Jak/Stat and preferentially activates ERK. Eosinophils are the only other leukocyte-type directly activated by IL-33, as evidenced by screening of p38-activation in peripheral blood cells. Only upon CD3/CD28-ligation, IL-33 weakly enhances Th2 cytokine expression by in vivo polarized Th2 cells. This study on primary human cells demonstrates that basophils and eosinophils are the only direct target leukocytes for IL-33, suggesting that IL-33 promotes allergic inflammation and Th2 polarization mainly by the selective activation of these specialized cells of the innate immune system.

OC9

The abundant CD56^{bright} NK-cell population after hematopoietic stem cell transplantation (HSCT) are cytokine activated – rather than immature natural killer cells

Vukicevic M., Chalandon Y., Helg C., Matthes Th., Dantin C., Huard B., Passweg J., Roosnek E.
Division of Hematology, Department of Internal Medicine, Geneva University Hospitals and University of Geneva, Switzerland

Approximately 95% of human peripheral blood (PBL) NK-cells expresses low levels of CD56 (NK56^{dim}) while only a minority is CD56-bright (NK56^{bright}). The relation between NK56^{dim} and NK56^{bright} is unknown. The abundance of NK56^{bright} after HSCT has been used as an argument that NK56^{bright} are immature precursors of NK56^{dim}. This reasoning is flawed because NK56^{bright} after HSCT differ considerably from NK56^{bright} in normal PBL. We have characterized NK-cells early after graft-take in 28 patients transplanted with an allogeneic graft most of which were T-cell depleted. We found that the number of NK56^{dim} was strictly correlated with the number of granulocytes ($R^2 = 0.4$, $p < 0.001$). By contrast, the number NK56^{bright} was neither correlated with the number of granulocytes nor with the number of NK56^{dim} ($p > 0.65$). Because we observed high numbers of NK56^{bright} only in patients with low numbers of T-cells, we tested the hypothesis that the abundant post-transplant NK56^{bright} were the progeny of classical NK56^{bright} that had expanded after stimulation by homeostatic cytokines like IL-15 for which they compete with T-cells. Indeed we found that after culture with IL-15 and SCF at concentrations comparable to those found in the sera of transplanted patients, NK56^{bright} from normal PBL proliferated vigorously, expressed CD11b and lost CD27 (maturity), lost CCR7 (lymph node homing), upregulated HLA-DR (activation) and perforin, downregulated c-kit and thereby became indistinguishable from the 'aberrant' posttransplant NK56^{bright} for all markers studied. Therefore we believe that the posttransplant NK-cell compartment is best described by the following paradigm: transplanted HSC initially produce granulocytes and NK-cells at a comparable low rate. Because IL-15 not competed for by T-cells preferentially stimulates NK56^{bright}, these cells acquire the 'aberrant' phenotype of posttransplant NK-cells. In addition, they expand to vastly outnumber the NK56^{dim} and reach the (supra)normal NK-cell numbers observed after HSCT.

OC10

A novel role for neutrophils as critical activators of natural killer cells

Spörri R., Joller N., Hilbi H., Oxenius A.
Institute of Microbiology, ETHZ, Biology, Zürich, Switzerland

Neutrophils are essential players in innate immune responses to bacterial infection. Despite the striking resistance of Legionella pneumophila (Lpn) to bactericidal neutrophil function, neutrophil granulocytes are important effectors in the resolution of legionellosis. Indeed, mice depleted of neutrophils were unable to clear Lpn due to a lack of the critical cytokine IFNγ which is produced by natural killer (NK) cells. We demonstrate that this can be ascribed to a previously

unappreciated role of neutrophils as major NK cell activators. In response to Lpn infection, neutrophils activate caspase-1 and produce mature IL-18, which is indispensable for the activation of NK cells. Furthermore, we show that the IL-12p70 response in Lpn-infected neutropenic mice is also severely reduced, and that the Lpn-induced IFNγ production by NK cells is strictly dependent on IL-12. However, since dendritic cells (DCs), and not neutrophils, are the source of Lpn-induced IL-12, its paucity is a consequence of the absence of IFNγ produced by NK cells rather than the absence of neutrophils per se. Therefore, neutrophil-derived IL-18, in combination with DC-produced IL-12, triggers IFNγ synthesis in NK cells in Lpn-infected mice. We propose a novel central role for neutrophils as essential IL-18 producers and hence NK cell "helpers" in bacterial infection.

OC11

Poly-I:C induces apoptosis in splenic dendritic cells

Fuertes Marraco S., Scott C.L., O'Reilly L., Bouillet P., Vremec D., Shortman K., Strasser A., Acha-Orbea H.
Université de Lausanne, Département de Biochimie, Epalinges, Switzerland

Clearance of activated dendritic cells (DC) is crucial to allow adequate control of immune responses. As a major group of professional antigen-presenting cells, DC are most potently activated by microbes and are pivotal in the induction of adaptive immune responses. While killing of DC has been described upon encounter with antigen-specific T cells, there is limited evidence on apoptotic mechanisms restricting DC lifespan in the event of microbial recognition. In this study, we report that PolyI:C, a synthetic dsRNA analog used as adjuvant, induces a profound depletion of mouse splenic DC in vivo, particularly affecting the CD8 DC subset. This observation could be extended to infectious settings involving viral components. Analysis of mRNA from sorted splenic DC following treatment in vivo reveals modulation of pro-apoptotic and anti-apoptotic genes. Most interestingly, this depletion is prevented in mice that are genetically deficient for the pro-apoptotic protein Bim in conjunction with a second Bcl-2 family member, either Puma, Noxa, or Bid. To our knowledge this is the first report of an apoptotic mechanism regulating splenic DC lifespan following treatment with a pathogen-associated molecular pattern (PAMP) in vivo. Failure to eliminate activated DC or steady-state DC accumulation can be disastrous to immune homeostasis and cause autoimmune disorders. These results may be particularly relevant to the understanding of sepsis and transient immune suppression upon infection, while the data could help in the optimization of DC-based vaccination strategies or therapeutic regimes where PolyI:C is included as an adjuvant.

OC12

Stimulation of dendritic cells via the dectin-1/Syk pathway allows priming of cytotoxic T-cell responses

Leibundgut-Landmann S., Osorio F., Brown G., Reis e Sousa C.
Institute for Microbiology, ETH Zürich, Zürich, Switzerland

The C-type lectin receptor dectin-1 functions as a pattern recognition receptor for β-glucans and signals via Syk kinase but independently of the Toll-like receptor (TLR) pathway to regulate expression of innate response genes. Dectin-1 signaling can promote activation of dendritic cells (DCs), rendering them competent to prime Th1 and Th17 responses. Here we show that dectin-1-activated DCs can also prime cytotoxic T-lymphocyte (CTL) responses. DCs exposed to a dectin-1 agonist induced antigen-specific expansion of TCR transgenic CD8+ T cells and their differentiation into CTLs in vitro. Dectin-1 agonist also acted as an adjuvant for CTL crosspriming in vivo, eliciting potent CTL responses that protected mice from tumor challenge. In vitro but not in vivo, CTL crosspriming was dependent on IL-12 p70, which was produced by dectin-1-activated DCs in response to IFNγ secreted by newly activated CD8+ T cells. The dectin-1/Syk pathway is thus able to couple innate immune recognition of β-glucans to all branches of the adaptive immune system, including CD4+ T-helper cells, B cells, and CD8+ cytotoxic T cells. These data highlight the ability of non-TLR receptors to bridge innate and adaptive immunity and suggest that dectin-1 agonists may constitute useful adjuvants for immunotherapy and vaccination.

OC13

Development of replication-defective lymphocytic choriomeningitis virus vectors for induction of potent CD8+ T cell immunity

Flatz L., Hegazy A.N., Bergthaler A., Verschoor A., Fernandez M., Kreppel F., Kochanek S., Löhning M., van den Broek M., Radbruch A., Lévy F., Nabel G.J., Lambert P.-H., Siegrist C.-A., Pinschewer D.D.
University of Geneva / C.M.U, Department of Pathology and Immunology, Geneva, Switzerland

Lymphocytic choriomeningitis virus (LCMV) exhibits natural tropism for dendritic cells and represents the prototype infection eliciting protective CD8+ T cell (CTL) immunity. Here, we have harnessed the immunobiology of this arenavirus for vaccine delivery. By utilizing producer cells constitutively synthesizing the viral glycoprotein (GP), it was possible to replace the LCMV-GP gene with vaccine antigens to create replication-defective vaccine vectors. rLCMV vaccines elicited CTL responses that were equivalent or greater than recombinant adenovirus 5 (rAd5) or recombinant vaccinia virus in their magnitude, cytokine profiles, and protective capacity. In contrast to rAd5, rLCMV failed to elicit vector-specific antibody immunity, thus facilitating re-administration of the same vector in booster vaccination. In addition, rLCMV elicited Th1 CD4 T cell responses and protective neutralizing antibodies against vaccine antigens. These features, together with its low seroprevalence in humans, suggest that rLCMV may show utility as a vaccine platform against infectious diseases and cancer.

OC14

Alveolar macrophages and lung dendritic cells sense RNA and drive mucosal IgA responses

Bessa J., Jegerlehner A., Hinton H.J., Schneider P., Bachmann M.F.
Cytos Biotechnology AG, Zürich-Schlieren, Switzerland

The mechanisms regulating systemic and mucosal IgA responses in the respiratory tract are incompletely understood. Using virus-like particles loaded with single-stranded RNA as a ligand for TLR7 we found that systemic versus mucosal IgA responses in mice were differently regulated. Systemic IgA responses were T-cell independent and did not require TACI or TGFb whereas mucosal IgA production was dependent on Th cells, TACI and TGFβ. Strikingly, both responses required TLR7 signaling but systemic IgA depended upon TLR7 signaling directly to B cells, while mucosal IgA required TLR7 signaling to lung dendritic cells and alveolar macrophages. Our data show that IgA switching is controlled differently according to the cell type receiving TLR signals. This knowledge should facilitate the development of IgA inducing vaccines.

Oral communications – Infectious diseases

OC15

Reassessment of recommended imipenem doses in febrile neutropenic patients with haematological malignancies

Lamoth F., Buclin T., Csajka C., Pascual A., Calandra T., Marchetti O.
Centre Hospitalier Universitaire Vaudois, Infectious Diseases Service, Lausanne, Switzerland

Imipenem is recommended for empirical therapy of febrile neutropenia. Plasma concentrations over the MIC of the causative pathogen during the whole dosing interval are recommended for optimal bactericidal activity in this life-threatening condition. Unpredictable variability of imipenem pharmacokinetics has been reported in critically-ill patients. The aim of this study was to assess the population pharmacokinetics of imipenem in neutropenic patients for optimizing dosage recommendations.
Methods: Imipenem plasma concentrations (peak and trough) measured by HPLC in 57 febrile neutropenic patients with haematological malignancies were analyzed using the NONMEM program. Based on the best model describing the data, simulations were performed to determine which dosage regimen would achieve a concentration exceeding the MIC90 of most common bacterias (1 mg/L) over the whole dosing interval.
Results: 159 imipenem plasma concentrations in 57 patients were analyzed. Imipenem volume of distribution was determined by body weight (Vd = 33.5 L for 70 kg BW) and clearance was correlated with glomerular filtration rate (CL = 15.5 L/h for 100 mL/min GFR, relative variability = 17%). Residual variability was 59%. According to the model, the recommended 500 mg q. 6h regimen achieved MIC90 coverage over the whole dosing interval in only 53% of patients with normal GFR. This goal could be achieved in 90% of patients with either 500 mg q. 4 h or 750 mg in 2-h infusions q. 6 h.
Conclusions: The recommended imipenem dose (2 g/d) frequently results in inappropriate plasma concentrations in febrile neutropenic patients. Higher doses (3 g/d) are needed to optimize drug exposure in this life-threatening condition.

OC16

Viral infections as a cause of chronic obstructive pulmonary disease (COPD) exacerbations

Rutschmann O.T., Kherad O., Janssens J.P., Sarasin F.P., Kaiser L.
Hôpitaux Universitaires de Genève, Service des Urgences, Genève, Switzerland

Introduction: Viruses are frequently isolated in patients with COPD exacerbations but their role as a trigger for these exacerbations remains debated.
Aim of the study: To describe the presence of viral infection during and outside COPD exacerbations.
Method: Prospective cohort study of 76 COPD patients admitted in the emergency department with acute exacerbation. RT-PCRs for 15 respiratory viruses were obtained by naso-pharyngeal swap at admission and 3 months after the index exacerbation. The presence of viruses was correlated with clinical characteristics and biological markers (CRP, procalcitonin) during exacerbation.
Results: 76 patients (mean age 72 y, male 64%) were included. During exacerbation, the following viruses were isolated in 38/76 (50%) patients: Picornarivus (n = 22), Metapneumovirus (n = 7), Coronavirus (n = 6), Influenza A/B (n = 2), Parainfluenza (n = 2), VRS (n = 2). A dual infection was present in 3 patients. Three months after the index exacerbation (follow-up available for 43 patients, ongoing study), viruses were identified in only 6/43 (14%) patients (p = .0002 compared to baseline): Picornarivus (n = 2), Metapneumovirus (n = 3), Parainfluenza (n = 1). In three of these patients, no virus was identified during the index exacerbation suggesting a new viral infection acquired during follow-up. During exacerbation, procalcitonin and CRP levels were not statistically different in patients with or without viruses identified. Time to clinical recovery and length of stay of the index hospitalization were similar in both groups.
Conclusions: Our results confirm the high prevalence of viral infections during COPD exacerbations. In contrast, viruses are rarely identified outside exacerbations suggesting that COPD exacerbations are frequently triggered by acute viral infections.

OC17

Eradication of extended-spectrum beta-lactamases (ESBL) producing enterobacteriaceae – a pilot study

Buehlmann M., Bruderer T., Bucher H.C., Frei R., Widmer A.F.
Infektiologie & Spitalhygiene, Kantonsspital Aarau AG, Aarau, Switzerland

Background: Extended-spectrum beta-lactamases (ESBL) producing Enterobacteriaceae are important emerging multiresistant pathogens. ESBL colonization can persist for years and likely favours ESBL spread. Decolonization (DC) for ESBL is not established. The aim of this study was to determine the effectiveness of a standardized DC regimen for ESBL eradication.
Methods: From 1/2000–1/2008, data of all ESBL carriers were prospectively recorded. Patients with ≥1 follow-up screening were included. ESBL infected patients were treated according to international guidelines. Persistent carriers were routinely screened by rectal, throat swabs and a urine sample before start of DC.
The DC regimen included: chlorhexidine 0.2% mouth rinses tid (throat colonization), paromomycin 4x1 g daily (intestinal colonization) and oral antibiotics for urinary tract colonization. ESBL eradication was defined as ≥1 set of negative screening cultures (throat, rectal, urine) and no further positive samples.
Results: 100 patients with ESBL were analyzed (83 infected, 17 colonized with ESBL). The most frequent pathogens were Escherichia coli (71%) and Klebsiella pneumoniae (25%). ESBL acquisition was nosocomial in 49%, health-care-related in 29%, and community-acquired in 22%. Urine was the most frequent initial site of ESBL infection or colonization. ESBL eradication was achieved in 51% (39/77) of patients after treatment of infection only. 35 patients underwent DC; 83% (15/18) of those completing DC were free of ESBL at follow-up (on treatment analysis).
Conclusion: The majority of patients became negative for ESBL at follow-up, mainly with systemic antibiotics for treatment of ESBL infection. DC of persistent ESBL carriers might be promising in a subgroup of patients.

OC18

Molecular evidence of interhuman transmission in an outbreak of *Pneumocystis jirovecii* pneumonia in renal transplant recipients

Gianella S., Haeberli L., Joos B., Ledergerber B., Wüthrich R.-P., Weber R., Kuster H., Hauser Ph.-M., Fehr T., Mueller N.J.
Division of Infectious Diseases and Hospital Epidemiology, University Hospital, Zurich

Introduction: *P. jirovecii* pneumonia (PCP) remains an important cause of morbidity and mortality in immunocompromised individuals. The epidemiology and pathogenesis of PCP is poorly understood and the exact mode of transmission remains unclear. Recent studies reported clusters of PCP among immunocompromised patients, raising the suspicion of interhuman transmission. We report an outbreak of PCP in renal transplant recipients (RTR) attending our nephrology outpatient clinic between 07/2006 and 02/2008.
Methods: An unexpected increase of PCP cases in our nephrology outpatient clinic prompted a detailed epidemiological and molecular analysis. Clinical data of 19 RTR with proven PCP infection were analyzed. Genotyping of 7 available specimens was performed using Multiple-Locus DNA Sequence Typing (MLST). Fragments of 4 variable regions of the *P. jirovecii* genome were amplified and sequenced (ITS1, 26S, mt26S, β-tubulin). The obtained sequences were compared with those of 4 independent control patients from different clinics of the same hospital.
Results: The nephrology outpatient clinic has its own waiting area not shared with other clinics. All but one of the 19 PCP-infected RTR had at least one simultaneous visit with another PCP-infected patient (mean of 5 common visits, range 0–14). PCP occurred between 3 months and 22 years after transplantation (mean, 48 months). No patient developed PCP while under prophylaxis. MLST analysis revealed identical sequences of the 4 regions among all 7 nephrology patients with available samples, indicating an infection with the same *P. jirovecii* type. The MLST types present in the 4 control patients were different from each other and differed from the type present in the 7 study patients in at least one of the 4 genomic regions.
Discussion: This study provides epidemiological and molecular evidence that nosocomial transmission of *P. jirovecii* among immunocompromised patients may occur. An environmental source cannot be firmly excluded. Our findings have potential epidemiological implications for the care of immunocompromized patients, and suggest that prolonged chemoprophylaxis for PCP may be warranted.

OC19

The relevance of CMV viremia, in the HAART era

Boffi El Amari E., Combescure C., Yerly S., Calmy A., Kaiser L., Hirschel B. and the Swiss HIV Cohort Study
Hôpital Cantonal Universitaire de Genève, Infectious diseases, Genève-14, Switzerland

Using new sensitive quantitative PCR's, CMV DNA is often detectable in the plasma of immunosuppressed patients who do not develop CMV disease. We study the value of a positive CMV DNA in predicting the development of an AIDS defining event or mortality, in patients with CD ≤100/μL, in the HAART era. Survival analysis of patients prospectively included in the Swiss HIV cohort Study, from January 1996 to July 2008, who were CMV seropositive, with a CD4 count ≤100/μL and a plasma sample available for the measurement of baseline CMV DNA, by an automated CMV real-time PCR (Abbot Molecular®), threshold of detection of 20 c/ml. Outcome analyzed: Aids defining event, CMV end organ disease, or death. Variables analyzed: age, sex, race, sexual orientation, CD4 counts, HIV-1 RNA concentration, use and type of HAART. Prognostic performance of CMV DNA measurement was assessed using time-dependant ROC curves. HR's were determined using a Cox model with a one year time horizon. Of 1170 patients, 208 presented an Aids defining event and 246 died (9.7 and 6.4% of patients respectively during the first 12 months). 34.4% of the total cohort had a positive baseline CMV DNA value, ranging up to 104000 c/ml. The optimal prognostic performance of the CMV DNA value in predicting an aids defining event was reached between 3 and 5 months (AUC of 0.679; 95%CI 0.568–0.777 and 0.662; 95%CI 0.578–0.736), whereas it was reached at 7 months for deaths (AUC of 0.614; 95%CI 0.520-0.694). A relevant cut-off value of CMV DNA was 80 c/ml. In the multivariate analysis, CMV DNA predicted not only the evolution towards CMV end organ disease (HR 12.5; 95%CI 4.20–37.25), but also towards other Aids defining events (HR 2.34; 95%CI 1.43–3.83) and death (HR 1.85; 95%CI 1.04–3.30). Quantitative CMV DNA detected early in the plasma of HIV patients with CD4 ≤100/μL, not only significantly predicts the evolution towards CMV end organ disease, but also towards other Aids defining events and death. A low value of 80 c/ml identifies patients at risk during the following months.

OC20

Comparison of two matrix-assisted laser desorption ionization – time-of-flight mass spectrometry methods with conventional phenotypic identification for routine bacterial speciation

Cherkaoui A., Emonet S., Tangomo M., Girard M., François P., Schrenzel J.
Hôpitaux universitaires de Genève, Laboratoire central de Bactériologie, Genève, Switzerland

Bacterial identification relies primarily on culture-based methodologies and requires 48–72 h to deliver results. Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as a new technology for bacterial speciation. By measuring the exact size of peptides and small proteins, rapid bacterial identification should become accessible to the routine diagnostic laboratory. In this study, we evaluated the performance of

two MALDI-TOF MS systems (MS) on 720 isolated bacterial colonies and in real routine conditions of work. Strains were analyzed in parallel on both devices, strictly relying on the manufacturers' default recommendations as well as by using conventional biochemical test systems. Whenever incongruent results were observed, 16S rDNA gene sequencing was performed. MALDI-TOF MS (Bruker) identified correctly 88% (634/720) of the isolates at the species level and 97% (695/720) at the genus level. The second device (Shimadzu) identified correctly 79% (568/720) and 93% (672/720) of the isolates to the species and the genus level, respectively. For the incongruent results, MALDI-TOF MS (Bruker) matched 27 of the 49 isolates (55%) to the sequencing results; of these, 14 were even identified at the species level. MALDI-TOF MS (Shimadzu) matched 19 of the 49 isolates (39%) to the sequencing results; of these, 9 were identified at the species level. Importantly, no single false identification at the genus level was generated by any MS method when relying strictly on manufacturers' default recommendations. Thus, MS-based species identification of bacteria provided accurate and reproducible results at very low reagent costs. Analytical modifications including improved extraction protocols for streptococci should further improve current identification yields and establish MS as a rapid and cost effective complement method to conventional phenotypic testing.

OC21

In infant rat pneumococcal meningitis, ceftriaxone plus daptomycin vs. ceftriaxone attenuates brain damage and hearing loss while ceftriaxone plus rifampicin vs. ceftriaxone does not

Leib S.L., Grandgirard D., Burri M., Oberson K., Bühlmann A., Simon F.
University of Bern, Institute for Infectious Diseases, Bern, Switzerland

Lytic antibiotics for therapy of bacterial meningitis (BM) increase the release of proinflammatory bacterial compounds which, in turns, induce inflammation. Exacerbation of the inflammatory response in cerebrospinal fluid (CSF) contributes to the development of neurological sequelae in survivors of BM. Daptomycin, a non-lytic antibiotic acting on Gram-positive bacteria has been shown to decrease inflammation and brain injury vs. ceftriaxone in experimental pneumococcal meningitis. With a view on the clinical application for empiric therapy of pediatric bacterial meningitis we investigated, whether therapies combining daptomycin or rifampicin with ceftriaxone are beneficial when compared to ceftriaxone monotherapy in infant rat pneumococcal meningitis.
Methods: Eleven day old Wistar rats were infected by intracisternal injection of S. pneumoniae and animals were treated with daptomycin (10 mg/kg, s.c., daily) plus ceftriaxone (100 mg/kg, s.c., bid), rifampicin (20 mg/kg, i.p., bid) plus ceftriaxone or ceftriaxone alone. CSF was sampled at 6 h and 22 h after the initiation of therapy and assessed for concentrations of chemo- and cytokines (MCP-1, MIP-1α, IL-1β, IL-6, IL-10; IL-18 and TNFα). A subset of animals was sacrificed 40 h post infection (h pi) and brain damage quantified by histomorphometry. The remaining animals were treated for 3 d and were tested for hearing loss, by assessing the auditory brainstem response (ABR) at 3 weeks after infection.
Results: Compared to ceftriaxone alone, daptomycin plus ceftriaxone significantly ($p < 0.04$) lowered CSF concentrations of MCP-1, MIP-1α and IL-6 at 6 h and MIP-1α and IL-1β at 22 h after initiation of therapy, led to significantly ($p < 0.01$) less apoptosis assessed at 40 h pi, and significantly ($p < 0.01$) improved hearing capacity. While rifampicin plus ceftriaxone also led to lower CSF inflammation ($p < 0.02$ for IL-6 at 6h), apoptosis and hearing capacity were not significantly different from the ceftriaxone group.
Conclusion: Compared to ceftriaxone monotherapy, daptomycin plus ceftriaxone lowers the level of pro-inflammatory mediators in the CSF and reduces hippocampal apoptosis and hearing loss in infant rat pneumococcal meningitis.

OC22

Efficiency of cross species infection depends on both HCMV tropism and porcine EC anatomical origin and is limited by apoptosis

Millard A.L., Häberli L., Ghielmetti M., Schneider M.K.J., Bossart W., Seebach J.D., Mueller N.J.
University Hospital Zurich, Department of Internal Medicine, Zurich, Switzerland

Reactivation of human cytomegalovirus (HCMV) is a potential risk following the clinical application of pig-to-human xenotransplantation. Therefore, we investigated the potential of various HCMV strains to infect porcine endothelial cells (pEC) in vitro. Human aortic (HAEC) and porcine EC from different anatomical origins were inoculated with various HCMV strains at multiplicity of infection (MOI) ranging from 0.1 to 30. Viral replication kinetics, development of cytopathology, release of viral progeny, and induction of apoptosis in HCMV infected pEC were analyzed. All virus strains infected pEC and maximal infection was reached at an MOI of 10 for AD169 and TB40/E and at an MOI of 1 for TB40/F. The fraction of infected cells ranged from 1% to 80%, depending on the MOI and the anatomical origin of the pEC. In TB40/F infected cultures, cytopathic effects were visible after 3 dpi, but occurred later in AD169 and TB40/E infected pEC. We also compared the permissiveness of pEC versus HAEC to HCMV infection. Even though late stage of infection was observed in both HAEC and pEC infected cultures, it was only reached in a minority of pEC showing a restriction of viral growth. The ability of HCMV infected pEC and HAEC to release viral progeny was evaluated in focal expansion assay. AD169 and TB40/F produced on pEC generated foci. Plaques from HCMV strains produced on pEC contained far fewer infected cells than plaque from HCMV strains produced on HAEC, confirming the restriction of HCMV growth in pEC. Finally, HCMV infected pEC demonstrated a 2-fold increased surface staining of annexin-V compared to non-infected pEC. Together, our data suggest that induction of apoptosis in HCMV infected pEC might be responsible for the reduced viral DNA replication and viral late gene expression observed.

OC23

Effects of cold shock of *Moraxella catarrhalis* on its interaction with host cells in vitro

Spaniol V., Troller T., Aebi C.
Institute for Infectious Diseases (IFIK), University of Bern, Bern, Switzerland

Moraxella catarrhalis, a major nasopharyngeal pathogen of the human respiratory tract, is exposed to substantial and rapid downshifts of environment temperature when humans breathe cold air. We investigated the effect of cold shock on the ability of M. catarrhalis strain O35E to adhere to, invade and induce an inflammatory response in human epithelial cells. Increased expression of UspA1, a major adhesin and putative virulence factor of M. catarrhalis, after cold shock at 26 °C, was a direct result of greater stability of mRNA, which was found to have a longer half-life than uspA1 mRNA exposed to 37 °C (3.0 vs. 1.8 min, $P < 0.0001$). Increased UspA1 expression after cold shock was correlated with both a 63% increase in binding to soluble fibronectin, an extracellular matrix component mediating cellular adherence, and enhanced bacterial cell-association, but not internalization, to Chang epithelial cells. Cold-shocked M. catarrhalis induced a significantly elevated release of proinflammatory mediator IL-8 in lung epithelial cells as compared to bacteria incubated at 37 °C. Similarly, a significantly enhanced proinflammatory response was observed when epithelial cells were stimulated with outer membrane proteins isolated from strain O35E exposed to 26 °C. A lipooligosaccharide (LOS)-deficient mutant strain induced significantly lower IL-8 secretion, and its cold shock response was less pronounced than in wild-type parent strain. Purified LOS, however, appeared to be a minor contributor to the stimulation of IL-8 by epithelial cells and its inflammatory properties were not influenced by cold shock. These data indicate that cold shock at a physiologically relevant temperature of 26 °C may affect the nasopharyngeal host-pathogen interaction and contribute to M. catarrhalis virulence.

OC24

Prognostic factors and JCV-specific immune responses in HIV-1 infected patients with progressive multifocal leukoencephalopathy from the swiss HIV cohort study (SHCS)

Elzi L., Khanna N., Wolbers M., Mueller N., Garzoni C., DuPasquier R., Fux C., Battegay M., Hirsch H.H.
University Hospital of Basel, Infectious Diseases and Hospital Epidemiology, Basel, Switzerland

Progressive multifocal leukoencephalopathy (PML) is a rare disease caused by uncontrolled JC virus (JCV) replication in the central nervous system. Despite combination antiretroviral therapy (cART) the prognosis of PML is poor. We defined primarily the incidence and prognosis of PML in all patients from the SHCS and secondly investigated in a subset of patients the JCV-specific cellular and humoral immunity.
Methods: All 159 PML patients with available CD4+ cell counts at diagnosis were included to analyze the incidence and prognostic factors. We compared pre-cART 1988–1995 and post-cART 1996-2007. Cox proportional hazard models were used to estimate the hazard ratios (HR) of PML-attributable death during the first year after diagnosis. A nested case control study was performed for JCV-specific immunity with 29 PML cases and 3 CD4 matched controls (n = 87) with available cryopreserved cell and plasma samples at diagnosis. Survivors (n = 18) were defined as being alive for >1 year after diagnosis.
Results: The PML incidence declined 4-fold after 1996 (0.24 [95% CI, 0.20–0.29] vs 0.06 [95% CI, 0.04–0.10] per 100 patient-years). PML-attributable 1-year mortality rate decreased from 82.3 [95% CI 58.8–115.1] to 37.6 [95% CI 23.–60.5] per 100 patient-years. By multivariate analysis, PML-attributable mortality was significantly reduced by cART

[p <.001; HR 0.15, 95% CI, 0.05–0.44], whereas all-cause mortality was associated with baseline CD4+ cell counts [p = .004; HR 0.35, 95% CI, 0.17–0.72] and cART [p = .013; HR 0.52, 95% CI, 0.31–0.87]. JCV-specific T-cell responses were lower in non-survivors than in controls (p = 0.08) which was significant for PCR or histology confirmed cases (p = 0.004). No difference was found between survivors and controls. Median JCV-IgG level was significantly higher in survivors than in non-survivors (p = 0.003) or controls (p = 0.007). The longitudinal analysis showed significant increases of JCV-specific T-cells (p = 0.04) and IgG response (p = 0.005) in survivors.
Conclusions: PML-attributable 1-year mortality has declined significantly by cART irrespective of baseline CD4+ cell counts. JCV-specific cellular immunity may be a critical determinant for 1-y survival. JCV-specific cellular and humoral immunity may serve as prognostic markers.

Posters – Allergology and immunology
Basic and clinical allergology

P1

Serumtryptase values during suspected systemic allergic reactions: even with normal maximal values a diagnostically relevant increase can often be documented

Borer-Reinhold M., Haeberli G., Bitzenhofer M., Hausmann O., Fricker M., Helbling A., Müller U.
Spital Netz Bern, Zieglerspital, Med. Klinik, Allergologie, Bern, Switzerland

A serum tryptase value above the normal limit of 11.4 ng/ml during an acute allergic reaction suggests release of mast cell mediators and thus of an IgE-mediated pathogenesis. However, an unequivocal increase of tryptase with maximal values below the normal limit might also be diagnostically relevant.
Patients and methods: We measured serum tryptase by UNICAP (Phadia, Uppsala Schweden) in 31 patients with suspected acute allergic reactions, after start of symptoms, after 2 hours, 5 hours, 24 hours and 3 days, in comparison with a baseline value before or at least 14 days after the reaction. Sixteen patients were hospitalized because of suspected acute allergic reactions. Fifteen patients developed allergic reactions during ultra-rush immunotherapy with hymenoptera venom or after a sting provocation test. Severity of allergic reactions was graded according to HL Mueller classification.
Results: Eleven patients had grade I–II reactions, 20 patients grade III–IV reactions. Hymenoptera venoms were the elicitors in 19 patients, drugs in 4, foods in 5, and immunotherapy injections of a pollen extract for seasonal allergic rhinitis in 3 patients. Twenty-one of the 31 patients (68%) showed an increase of serum tryptase to more than 150% of their baseline value at start of symptoms or within 5 hours thereafter, 19 even an increase to more than 200%. Only 13 of all 31 patients (42%), with an unequivocal increase (to >150%) showed a maximal serum tryptase value over 11.4 ng/ml during the allergic reaction. An unequivocal increase was documented in 16 of 19 cases due to bee- or yellow jacket venom, 2 of 5 to foods, 2 of 4 to drugs and 2 of 3 to pollenextracts.
Conclusion: Analysis of a suspected systemic allergic reaction by estimation of serum tryptase should always include a baseline value, even in the presence of maximal values in the normal range during the acute phase.

P2

Role of regulatory T cells in the induction of tolerance in a murine model of asthma

Boudousquié C., Pellaton C., Barbier N., Spertini F.
Centre Hospitalier Universitaire Vaudois, Immunologie et allergie, Lausanne, Switzerland

Rationale: Regulatory T cells (Tregs) are key players in controlling the development of asthmatic inflammation. The respective role of different Tregs subsets in the induction of allergen specific tolerance in established asthma has not yet been elucidated.
Methods: BALB/c mice were sensitized and tolerized to ovalbumin (OVA). Induction of Tregs in tolerized mice was analyzed by flow cytometry. To assess suppressive capacity and proliferation in vivo, CD4+CD25+, CD4+CD25- or CD4+CD25-mTGFβ- T cells were purified from asthmatic or tolerized donor mice and transferred into recipient asthmatic mice. Cytokine secretion profiles were determined in vitro.
Results: Tolerization led to a marked upregulation of regulatory CD4+CD25+Foxp3+ T cells in the lungs. In vivo transfer of CD4+CD25+ T cells had no effect on lung inflammation whereas transfer of CD4+CD25- T cells led to reduced eosinophils recruitment upon challenge. Interestingly, CD4+CD25- T cells transferred from asthmatic mice treated intranasally with OVA but depleted of CD4+CD25+ T cells had no effect on eosinophils numbers in the lungs of recipient mice. Furthermore, the transfer of CD4+CD25-mTGFβ- T cells suppressed eosinophilic inflammation but less efficiently than CD4+CD25- T cells. PKH26 labeled CD4+CD25- T cells did not proliferate in vivo. When stimulated with OVA in vitro, CD4+CD25- T cells produced high amounts of IL10, MCP1, low IL-5 and no TGFβ, IL-17 or IFNγ.
Conclusions: CD4+CD25+ and CD4+CD25- regulatory T cells both appear to be essential in tolerance induction. The relationship between subsets and the role of CD4+CD25-mTGFβ+ T cells will have to be further analyzed.

P3

Snack seeds allergy in children

Caubet J.-C., Wassenberg J., Hofer M.-F., Eigenmann P.-A.
Allergologie pédiatrique, Hôpitaux Universitaires de Genève, Genève 4, Switzerland

Introduction: Snack seeds, particularly sunflower and pumpkin seeds, are popular and often consumed by children. Both are also used for cooking. Instead of their frequent consumption, only a few cases of anaphylactic reactions in adults are reported in the literature.
Cases-report: We report 3 cases of snack seeds allergy in children. The first two children developed a severe anaphylactic reaction few minutes after eating a snack containing sunflower seeds. Both of them required treatment with anti-histamines and corticosteroids and one of them required in addition epinephrine in the emergency unit. One of them had a positive prick-to-prick test with sunflower seed, whereas the other had a negative one. Specific sunflower seeds IgE were found by CAP-RAST in these 2 patients (respectively 0.51 kU/l and 3.48 kU/l). The oral provocation tests, considered as the gold standard, confirmed the allergy. The last patient, an 11-year old boy, presented an anaphylactic shock few minutes after he ate pumpkin seeds. The prick-to-prick test with pumpkin seeds was negative but an oral provocation test confirmed the allergy. All the patient required corticosteroids and anti-histamines after the oral provocation test. The last patient, with pumpkin allergy, required also epinephrine because of a particularly severe reaction with urticaria, angioedema and asthma.
Conclusion: Pumpkin and sunflower seeds allergy is unusual, especially in children. Due to the severity of the reactions presented by our patients and the frequent use of this seeds as snacks or for cooking, sunflower and pumpkin seeds allergy should be evaluated in case of snack seeds allergy suspicion or even after idiopathic anaphylaxis. Due to the poor sensitivity of skin tests, a complete allergology work-up, including oral provocation tests, should be considered.

P4

1 to 3 years delay between sensitization and first symptoms in ragweed allergy

Davet A., Clot B., Gumowski P.I.
INRAAIC, Allergy & Clinical Immunology Research Institute, Meyrin-Genève, Switzerland

Objectives: A study on the neo-sensitizations to ragweed in the Geneva area is ongoing since 1999. In a previous analysis a discrete but regular increase in the number of the neo-sensitizations was observed (SSAI 2007). In this new study, the intervals between the first positives tests, the onset of the first symptoms and the pollinic indexes are analysed.
Methods: Files of 1509 new patients [NP] were reviewed: NP with respiratory symptoms: 838; NP with late summer symptoms (August and September) [LSS]: 211; NP with demonstrated sensitization: 56 [DS].
Results: A correlation between positives Prick-tests [PrT] and the specific-IgE [sIgE] for ragweed and the quantity of ragweed pollen during the previous year is observed; i.e.: between the results of tests in new patients recruited between August 2000 and July 2001 and the cumulated pollens and pollen index for ragweed in 1999.1) Likewise a similar correlation appears to exist between the quantity of ragweed pollens from the previous year and the number of new patients symptomatic the following August and September [LSS]. The same is observed among patients specifically sensitized to ragweed [DS]. 2) A similar association appears also at 3 years, suggesting a possible cumulative effect. 3) Conversely, a reduction in the number of new symptomatic patients [LSS] is observed the year following a lower pollinization: i.e.: a decrease in the number of new symptomatic patients at the end of the summer 2004 can be seen following a 2003 summer with rather low amounts of ragweed pollens measured. This impression is comforted by the follow-up of sensitized patients (newly

diagnosed with PrT and/or sIgE +), but asymptomatic at the moment of the tests, that develop symptoms to ragweed pollen exposure 1 or 2 years later. In most of these patients, the apparition of specific symptoms is associated with a regular increase of ragweed sIgE measured from one year to the other.
Conclusion 1: The data collected since 1999 point to show that, in exposed subjects, the development of positive biological and/or clinical tests can precede up to 1 to 3 years the first manifestations of ragweed allergic symptoms.
Conclusion 2: Though these results would need to be corroborated by a larger prospective study, they clearly put in evidence the need of a strong control of ragweed extension to limit the occurrence of a sensitization and the subsequent clinical manifestations.

P5

Effect of oral Broncho-Vaxom® on nasal challenge with grass pollen in patients with allergic rhinitis

Gambillara E., Wassenberg J., Audran R., Barbier N., Pigache-Renard K., Schmidt M., Cozma G., Spertini F.
Service d'immunologie et d'allergie, CHUV, Lausanne, Switzerland

Background: Oral administration of bacterial extracts (Broncho-Vaxom®, BV) was able to switch a Th2 type immune response towards a Th1 type in animal models suggesting the potential for BV to prevent allergy symptoms. We thus investigated the effect of one-month oral administration of BV on a nasal provocation test (NPT) with grass pollen in adults with allergic rhinitis.
Method: We performed a randomized, placebo-controlled, double-blind parallel-group study including 60 subjects with a history of seasonal allergic rhinitis to grass pollen. The study design involved an initial NPT at inclusion, a treatment phase of 30-days with a second NPT at day 29 and a final visit at day 30. NPTs were performed in a dose-escalation manner during the out-of-season period; immunological parameters were analyzed from nasal samples, serum and PBMC, before, during and after NPTs.
Results: Differences in the mean allergen dose level (primary end point) necessary to reach the global NPT threshold indicated a non-significant trend toward improved protection provided by BV as compared to placebo (median shift from 1000 to 3300 SQ/mL after BV *versus* 1000 to 1000 SQ/mL after placebo). No significant change in threshold for individual parameters of NPT (sneezes, nasal secretions, MCA (minimal cross-sectional area), PNIF (peak nasal inspiratory flow), VAS (visual analogue scale)) was shown in the treated compared to the placebo group. In nasal samples, albumin decreased after BV ($p = 0.036$) as well as ECP ($p = 0.02$), and IL-5. In supernatants from PBMC stimulated with grass pollen, IFNγ was enhanced ($p = 0.02$).
Conclusion: These results demonstrate that a short term treatment period with BV compared to placebo was able to downregulate Th2 markers of the nasal and systemic proinflammatory response in a provocation test with allergen. Exudation of albumin, a robust marker of allergic inflammation, into nasal secretion was limited after BV treatment. Furthermore, there was a trend toward improvement of global clinical score of NPT. This suggests that BV may contribute to improve established seasonal allergic rhinitis. Further longer-term seasonal studies are warranted.

P6

Double positivity in insect venom allergy – diagnostic approach with basophil activation test

Hausmann O., Gentinetta T., Schneider M., Helbling A., Pichler W.J.
Allergologisch-Immunologische Poliklinik, Rheumatologie und Klinische Immunologie/Allergologie, Inselspital, Bern, Switzerland

Introduction: In spite of a clear-cut history, standard diagnostic work up in insect venom allergy (skin tests, serology) reveals double-positivity to honey bee and wasp venom in up to 40% of patients. Cross-reactive carbohydrate determinants (CCD) were in part held responsible for this phenomenon, but they mostly lack clinical relevance. As the CAP technique (CCD-specific IgE, CAP inhibition assays) did not prove to be helpful in this respect, we studied the usefulness of a basophil activation test (BAT) to differentiate between crossreactivity and allergy to both insect venoms.
Method: 9 patients with a history suggestive of wasp venom allergy and double-positivity in intradermal testing and/or serology were recruited. 16 bee venom allergic patients and 11 healthy, stung controls were included for determination of positivity thresholds (ROC analysis). We used commercially available basophil test kits (Flow2CAST, Buhlmann Laboratories) with modified test conditions (whole blood/anti-CCR3/anti-CD63 ± IL3) and varying venom concentrations (2 to 285 ng/ml, final concentration). Blood samples (EDTA) were analyzed within 24 h of blood sampling (stored at 4 °C if not analyzed within 4 h).
Results: ROC analysis revealed concentration-dependent positivity thresholds for bee venom between 2.5–3.5% (IL-3-free condition) and 5–10% (with IL-3) regarding CD63 upregulation. In 4/9 patients a wasp venom allergy could be established, whereas double positivity was confirmed in the remaining 5 patients. Determination of CCD specific IgE (Bromelain) was positive in 3/9 patients, all turning out double positive in the BAT assay.
Conclusion: BAT helped in establishing diagnosis in almost half of the patients (4/9) which correlated well with history and skin test results (higher sensitivity to wasp venom). The highest (285 ng/ml) and the lowest venom concentrations (2 ng/ml) were not helpful in discriminating between the two patient groups and can therefore be omitted. CCD positivity did not exclude double positivity in the BAT assay and therefore did not prove to be helpful. BAT may be helpful for clarifying the relevance of double positivity in insect venom allergy and is less time consuming than CAP inhibition.

P7

Intranasal and intragastric administration of *L. paracasei* NCC2461 inhibits respiratory allergic response in mice and induces regulatory T cells

Pellaton C., Nutten S., Boudousquié C., Thierry A-C., Barbier N., Kierstein S., Mercenier A., Corthésy B., Spertini F.
Centre Hospitalier Universitaire Vaudois, and Nestlé Research Center, Lausanne, Switzerland

Background: Preclinical and clinical evidences for a role of probiotics in the management of allergic diseases are emerging.
Objective: To test the immunomodulatory effects of oral *versus* intranasal administration of *L. paracasei* NCC2461 in a mouse model of respiratory allergy and to investigate mechanisms of regulation induced by NCC2461.
Methods: Mice were sensitized intraperitoneally with ovalbumin (OVA) and challenged with OVA aerosols. *L. paracasei* NCC2461 or *L. plantarum* NCC1107 strains were administered between aerosol challenges either intragastrically (NCC2461) or intranasally (NCC2461 or NCC1107). Inflammatory cell recruitment into bronchoalveolar lavage fluid (BALF), eotaxin and IL-5 production in the lungs and OVA-specific IgE production in sera were measured post-aerosol challenges. Regulatory T cell subsets from lung extracts were evaluated by flow cytometry.
Results: Both oral and nasal administrations of *L. paracasei* NCC2461 efficiently protected sensitized mice against inflammatory cell recruitment into the bronchoalveolar lavage fluid (BALF) upon OVA aerosol challenges, and inhibited OVA-specific IgE production as compared to control mice. Eotaxin and IL-5 secretion in the lungs was also down-regulated. Intranasal administration of NCC2461 induced a stronger and more reproducible down-modulation of the allergic response than intragastric application, and induced an increase in $CD4^+CD25^+Foxp3^+$ regulatory T cells in the lungs. None of these effects were observed when mice were given *L. plantarum* NCC1107 intranasally as control.
Conclusion: *L. paracasei* NCC2461 administered intranasally or orally specifically display anti-allergic effects in a mouse model of respiratory allergy. This effect may be due, at least in part, to the induction of regulatory T cells.

P8

Identification and characterization of Asp f34 a novel major allergen of *A. fumigatus*

Rhyner C., Glaser A.G., Kirsch A.I., Zeller S., Menz G., Crameri R.
SIAF, Molecular Allergology, Davos Platz, Switzerland

Despite the fact that fungal spores are a well documented cause of allergy and asthma and the first components of aerial exposure, only two allergenic fungal cell wall components have so far been described: a minor allergen of *Cladosporium herbarum* and a major allergen of *Malassezia sympodialis*. Highthroughput screening of *A. fumigatus* cDNA libraries displayed on phage surface revealed at least 81 sequences potentially encoding IgE-binding proteins. They were examined in silico for the presence of cell wall components. The cDNA encoding a protein formally termed Asp f 34 spans an open reading frame predicting a protein of 185 amino acids with a molecular weight of 19.4 kDa showing sequence homology to phiA, an essential protein for the formation of conidia in the genus *Aspergillus*. Recombinantly produced Asp f34 showed binding of serum IgE of sensitized individuals in Western blots and ELISA and allergen specific T cell proliferation exclusively in patients sensitized to the allergen. An ELISA survey showed that 94% of the patients with allergic bronchopulmonary aspergillosis (ABPA) and 46% of the other *A. fumigatus*-sensitized individuals tested, had Asp f 34-specific serum IgE. The in vivo relevance of Asp f 34 was demonstrated by skin prick tests where only patients with detectable anti-Asp f 34 serum IgE scored positive, whereas *A. fumigatus*-sensitized individuals without Asp f 34-specific IgE and healthy controls scored negative. These results demonstrate Asp f 34 being a major allergen of *A. fumigatus*. Because proteins of the phiA family are found exclusively in the genus *Aspergillus*, Asp f 34 has relevant potential for a specific diagnosis of *Aspergillus* sensitization and for the detection of ABPA, the most severe pulmonary complication related to fungal allergy.

P9

ASS desensitisation in widal reaction: a retrospective analysis of data

Rothe T., Jung H., Günter G.
Klinik für Pneumologie und Allergologie, Hochgebirgsklinik Davos, Davos Wolfgang, Switzerland

Chronic sinusitis with nasal polyps often complicates asthma and may be associated with intolerance to ASS (Morbus Widal). Desensitisation to ASS is a promising approach to reduce nasal symptoms by preventing growth of polyps and improving sense of smell. In a retrospective analysis in December 2008 we tried to contact by phone all 35 patients having been desensitised between July 2007 and September 2008 with ASS in our clinic. 29 could be interrogated allowing us to fill in a questionnaire including length of ASS-treatment, dosage, nasal symptoms, and reasons of an eventual discontinuation of therapy. The mean duration of ASS treatment at that very moment was 7.9 months (4 to 15). 23 patients (79%) acknowledged an improvement of nasal symptoms. 17 had less symptoms of the upper airways and 15 reported an improvement of their sense of smell. 18 patients were still on ASS therapy. The main side effects were hematoma (5) and gastric symptoms (4). 11 patients had stopped ASS desensitisation: 2 due to gastric symptoms, 4 due to scheduled operations, 3 due to subjective ineffectiveness, and 1 due to asthmatic exacerbation. Severe side effects did not occur. Discontinuation of therapy occurred always within the first 6 months of treatment. We therefore conclude that after 6 months of treatment with ASS the success rate can be judged already. ASS desensitisation is a secure and effective approach to cure nasal polyps in Widal reaction. Thorough instruction of patients and their practitioners about this specialised therapeutic option may help to improve the potential benefit. Interestingly, 62% of our patients being desensitised with ASS in our clinic were atopic. This number is much higher than the 30% the AIANE investigators had found in Europe.

P10

Diagnostic of anaphylactoid reactions to opiates

Scherer K., Branka L., Grize L., Bircher A.J.
University Hospital Basel, Allergy Unit, Department of Dermatology, Basel, Switzerland

Introduction: Opiates, such as morphine, codeine and synthetic opioids such as pethidine and tramadol can cause mast cell degranulation without the presence of specific IgE antibodies. This effect is enhanced by the heterogeneity of mast cells with a different perceptibility to the histamine-releasing stimulus of e.g. morphine depending on the localization. These phenomena cause difficulties in the differentiation between sIgE-mediated and pharmacological mast cell mediator release in the allergologic work-up of opiate-induced anaphylactoid reactions. In this study, we investigated the skin test reactivity to different opiates in healthy volunteers.
Methods: 16 healthy volunteers without prior reaction to opiates were investigated with intradermal skin tests (IDT) using different concentrations of codeine, morphine, fentanyl, pethidine and tramadol. Histamine and NaCl 0.9% served as controls. An image of each weal was taken after 15 min. using a digital camera equipped with an optical device for direct skin contact with integrated scale. The digital images were then analysed using the NIH Image J software version 1.3 (freeware). After standardization to the integrated scale, the circumference of the weal was determined in triplicate and the area was calculated. Dose response curves were calculated for each substance and volunteer and normalized to the negative control. Threshold concentrations were determined to differentiate between true and false positive IDT.
Results: Skin test reactivity differed greatly between the different subjects and substances. It was still possible to determine threshold concentrations for each substance with a reasonable confidence interval although the situation is probably more accurately described by determining a threshold interval of positivity for each drug considering the individual skin test reactivity.
Conclusion: The newly defined threshold intervals for positive tests with different opiates in healthy volunteers will improve skin test reliability in the allergologic work-up of anaphylactoid reactions to opiates.

P11

Severe intraoperative anaphylaxis in a patient sensitized to latex and ethylene oxide: management of further surgery

Scherer K., Frei F.J., Hasler C.C., Spörl D., Bircher A.J.
University Hospital Basel, Allergy Unit, Department of Dermatology, Basel, Switzerland

Introduction: Sensitization to latex is common in patients with myelomeningocele. Ethylene oxide (EO), a highly reactive alkylating compound, is widely used as a sterilisation gas for various heat-sensitive medical devices (MD). EO-sterilization of dialyzers was discontinued due to widespread hypersensitivity to EO in dialysis patients. sIgE to EO has been shown to occur in 23% of patients with myelomenigocele with unknown clinical relevance.
Patient: We present a 13 year old male patient with myelomeningocele and known latex sensitization, who had undergone several previous unproblematic latex-free surgeries. During yet another spine surgery for skoliosis, he developed a prolonged anaphylactic reaction with severe hypotension, urticaria, angioedema and mast cell tryptase of >100 ug/l. Allergologic workup showed type I sensitization to EO (sIgE to EO 89 kU/l) and latex. All other drugs used during anaesthesia were tested negative repeatedly using skin tests and if possible sIgE. Two further extended spine surgeries were indicated.
Results: The patient received biweekly Omalizumab for 3 months. If possible EO-free devices were procured. Many MD were only available sterilized with EO. The attempt to prerinse these MD to minimize EO-content was not controllable due to technical reasons. All EO-MDs were therefore plasma-sterilized. During the next surgery he developed a milder reaction with hypotension and flush despite premedication with antihistamines. The EO and latex-free conditions were reassessed and Omalizumab was continued. 2 and 3 weeks later the patient underwent two further surgeries without any problems.
Conclusion: Our patient is sensitized to both a hapten (EO) and a protein (Latex). Although there are some similar cases in the literature there is little information on the exact measures to ensure an EO- and latex-free surgical intervention. Additionally Omalizumab may be helpful in preventing anaphylactic reactions to traces of EO or latex.

P12

A delicious but dangerous raspberry (*Rubus ideaus*) jelly jam from a 68 year non atopic grand mam having presented a lipid transfer protein (Rub i3) mediated anaphylactic reaction

Schneeberger D., Barbey J.L., Déruaz C., Hammann C., Mantegani A., Fellrath J.M., Kaeser P., Pastore F., Weilenmann C., Ballmer-Weber B.K.
Private practice, allergology and clinnical immunology, Fribourg, Switzerland

Up to 50% of birch pollen allergic patients react mildly (by the so called oral allergy syndrom) when eating fruits of the *Rosaceae* family (mainly apples, pears, peaches, rarely also berries), nuts and vegetables due to a sensitisation to Bet v 1 homologous or profilin epitopes. More severe anaphylactic reactions are reported – in some patients (mainly Spanish) – after consumption of peach for example, related to a heat stable allergenic protein called lipid transfer protein (LTP: Pru p3) an ubiquitous plant food lipid binding protein and potential pan-allergen. We here report an anaphylactic reaction in a 68 year old lady after ingestion of cooked raspberry jelly jam.
History: Five minutes after eating raspberry jelly jam this 68 old patient – treated for a metabolic syndrom – presented palmoplantar pruritus, generalized urticaria and an enormous lip angioedema needing emergency out hospital treatment by i.v. corticosteroid and i.v. antihistamine. 10 years ago she presented a similar but weaker reaction after raw bilberry consumption. She tolerates well peaches and apples.
Allergic workup: revealed strong positive skin reactions to defrozen raspberry (wild and common) and bilberry extract, and cooked raspberry jelly jam whereas all other skin tests (common aeroallergen and food allergens) resulted negative. Specific IgE (CAP: Phadia®) to recombinant peach LTP (rPru p3 = f420) was increased with 1,64 kU/l (class 2), to whole raspberry (f343) with 0,84 kU/l, and to bilberry with 0,37 kU/l (f288). Ig E to Bet v 1 (t215), however, were negative.
Conclusion: this case of a raspberry induced anaphylactic reaction by highly processed raspberry products underlines the distinct heat stability of the raspberry LTP (Rub i 3). Our diagnosis of a LTP mediated allergy to raspberry is supported by the strong positive skin test with heated raspberry jelly jam and the positive in vitro test to recombinant peach LTP. Due to a high cross-reactivity among fruit LTPs, peach LTP might be applied as a screening protein for LTP sensitisation. Further work to better identify and characterize raspberry (and other berries) allergens, in particular the LTP (Rub i3) is warranted.

P13

Not food associated exercise induced anaphylaxis (FAEIA) but wound disinfectant application induced anaphylaxis (WDAIA) in a 34 years atopic football player

Schneeberger D., Barbey J.L., Lachat J.M, Mantegani A., Déruaz C., Hammann C., Fellrath J.M., Pastore F., Weilenmann C., Gamba M.A., Kaeser P.
Private practice, allergology and clinnical immunology, Fribourg, Switzerland

Background: FAEIA is an uncommon but not exceptional cause of anaphylaxis during exercise. Disinfectants – such as chlorhexidine (CHX) – are together with other compounds – like dyes – rarer causes of perioperative anaphylaxis. Chlorhexidine – available as OTC solutions – is widely present in many homes. Nothing is known about the prevalence of CHX hypersensitivity in atopic or non atopic humans.

We report the case of a young atopic football player who presented an anaphylactic reaction when he applied an antiseptic solution (Bepanthen spray®) after the match.
History: A 34 years man after having eaten cornflakes with milk and some bred at breakfast played during 1½ hour football. One hour after he stopped the game he applied a disinfectant (Bepanthen spray®) on a wound of his right knee contracted during the match. Ten minutes later he presented generalized pruritus, urticaria with Quincke oedema needing – in the emergency outpatient room – i.v. corticosteroid and antihistamine treatment.
Allergic workup: Atopic status in the patient was supported by positive skin tests for grass and tree pollen (hazel, ash) in relation with a slight seasonal rhinoconjunctivitis. All food allergens tested (wheat in particular) were negative whereas Bepanthen Plus® spray and chlorhexidine revealed strongly positive. The other components of the spray were negative. Total IgE amounted 14.7 kU/l. Specific IgE (CAP: Phadia®) were positive for chlorhexidine (c8) at 1,22 kU/l in class 2 and for grass pollen (gx3) at 4,34 kU/l in class 3. Serological analyses for all food allergens (wheat, rye, celery, peanut, omega-1-gliadine) were negative. Tryptase level was normal at 6,1 ug/l.
Conclusion: CHX an ubiquitous disinfectant – identified as emerging factor of perioperative anaphylaxis with known risk factors such as male gender, older age, previous mild reaction on prior exposure and urologic surgery – is a potential anaphylactogenic agent, but probably often overlooked. CHX has thus to be considered in allergic workup as cause of anaphylaxis even outside the hospital setting, particularly during wound treatment procedures. Strong positive skin test with the whole disinfectant spray, CHX alone, positive serological testing for CHX together with the exclusion of FAEIA allowed firm diagnosis of WDAIA in our case. Further epidemiological and clinical studies in larger patient samples are needed.

P14

Absence of new sensitization to the non-culprit not treated insect venom during venom immunotherapy

Spörl D., Bircher A.J., Scherer K.
University Hospital Basel, Allergy Unit, Department of Dermatology, Basel, Switzerland

Venom immunotherapy (VIT) has proven to be efficacious in reducing the severity of anaphylactic reaction following field sting in hymenoptera venom allergy. Since no recombinant allergens are yet available, standardized vaccines containing different allergens are used in immunotherapy. Due to sequence homologies among allergens in vaccines for allergies to different hymenoptera, there is concern that immunotherapy could lead to sensitization to allergens to which patients were not sensitized before. This has been reported in the literature but the incidence and relevance of such an undesired phenomenon is unclear.
Methods: The aim of our study was to investigate the incidence of new sensitization to hymenoptera venoms during VIT other than those to which the patients were already sensitized. This was done by a retrospective analysis of specific immunoglobulin E (sIgE) in patients with no prior detectable sIgE to hymenoptera other than the one for which they received VIT.
Results: Of the 56 patients who had VIT, three (5%) showed development of prior undetectable sIgE to the other insect with no history of field sting which could explain it. This rate was similar to the rate of new sensitization due to field stings during VIT. No patient had a systemic anaphylactic reaction after having been stung by an insect other than the one he was desensitized for during the follow up period.
Conclusion: VIT seems to be safe and not to cause clinically significant new sensitization.

P15

IgE mediated anaphylaxis to an intra-articular glucocorticoid preparation proven by basophil activation tests

Steiner U.C., Gentinetta Th., Hausmann O., Pichler W.J.
Allergologisch-Immunologische Poliklinik, Rheumatologie und Klinische Immunologie/Allergologie, Inselspital, Bern, Switzerland

Glucocorticoid preparations rarely trigger immediate allergic reactions. These may be due to an allergy or "pseudoallergy" to excipients and rarely to the glucocorticoid preparation itself. Usually, diagnosis is based on history and skin prick tests (SPT), but basophil activation test (BAT) were not yet used.
Case 1: A 67-year-old man received an intra-articular infiltration L4/5 with Kenacort® A 40, Bupivacain® and the contrast media Iopamiro® to better localize the inflitration. After 15 minutes the patient developed generalized itching, urticaria, angioedema, dyspnea and dizziness. The patient recovered in the intensive care unit after administration of fluids, methylprednisolone and antihistamines.
Case 2: A 54-year old man experienced an anaphylactic reaction with generalized erythema, facial angioedema and dyspnea, 5 minutes after an intra-articular facet-joint infiltration with Kenacort® A 40, Bupivacain® and Iopamiro®. The patient quickly recovered after infusions of fluids, methylprednisolone and antihistamines.
Diagnosis: In both patients SPT with Kenacort® were positive, but SPT to the isolated components of Kenacort® suspension (Triamcinalone acetonide, CMC and Polysorbate 80) and Bupivacain® and Iopamiro® remained negative. The BAT showed in both patients a strong, dose-dependent upregulation of CD63 and of CD203c to Kenacort® suspension and to CMC, but not to other substances. Moreover, passively sensitizing third party basophils with the serum of the patients made them reactive to CMC, demonstrating IgE as transferable serum factor.
Conclusion: The data show that a) immediate reactions to glucocorticoid suspensions are often due to the excipients, in particular CMC; b) that the BAT is superior to SPT in differentiating the reactivity to the isolated components of the glucocorticoid suspension; and c) that the BAT after passive sensitization of third party basophils with patients serum could even prove the IgE mediated nature of the anaphylaxis to CMC.

P16

Age-dependency of sting recurrence in children with hymenoptera venom immunotherapy: higher prevalence of re-stings but fewer systemic reactions in younger children

Stritzke A., Eng P.A.
Kantonsspital Aarau, Allergology, Aarau, Switzerland

Hymenoptera venom allergies in young patients are of great concern because children tend to be more prone to re-stings than adults.
Objective: This study aims to determine the age-dependency of sting recurrence and subsequent systemic allergic reaction (SR) in children with anaphylaxis to hymenoptera venom after commencement of immunotherapy (VIT).
Methods: 83 children with grade III or IV allergy to bee (n = 49), wasp (n = 29) or both hymenoptera venoms (n = 5) were retrospectively followed-up via file and telephone survey. Mean follow-up period was 7.7 years after commencement of VIT. Group 1 included preschool children (<6 years, n = 17), group 2 school children (6–10 years, n = 39) and group 3 young adolescents (>10–16 years, n = 32). Endpoints were rate of re-stings and percentage of SR to re-stings in relation to age.
Results: 49 children (59%) had been re-stung 108 times by the insect they were allergic to, averaging in 1.2 stings per patient. There was no difference between prevalence of bee and wasp stings. However, younger children were being stung significantly more often than older children: the rate of re-stings per year was 0.41 in group 1 (preschool age), 0.21 in group 2 (school age) and 0.15 in group 3 (adolescents) respectively. Regression analysis showed a significant age dependency in prevalence of re-stings ($p = 0.001$). SR upon re-sting were fewer in younger children with 1/29 stings (3%) in group 1, 2/47 stings (4%) in group 2, and 5/32 stings (15.6%) in group 3. Differences in prevalence of SR were significant ($p < 0.05$) between preschool and school children (group 1 und 2) as compared to adolescents (group 3). Overall, there was a trend for higher prevalence of SR upon re-stings with increasing age ($p = 0.079$).
Conclusions: A majority of children with hymenoptera venom allergies are being re-stung. The younger the patients, the higher the prevalence of re-stings. In younger children however, recurrence of systemic reactions upon re-stings is lower as compared to adolescents.

P17

On the regulation of IgE-dependent mast cell activation

Uermösi C., Manolova V., Beerli R., Bauer M., Bachmann M. F.
Cytos Biotechnology AG, Schlieren, Switzerland

Atopic reactions are symptomatic responses to a normally harmless environmental antigen, due to excessive production of antibody of the immunoglobulin (Ig) E class. In contrast to other immunoglobulin classes, such as IgM, IgG and IgA, which circulate in the blood and lymph, allergen-specific IgE is most prominent in epithelia and mucosa where it is bound to the high-affinity Fcε; receptor I (FcεRI) on mast cells, basophils or activated eosinophils. Cross-linking of receptor bound IgE by its cognate antigen is a key trigger for type I hypersensitivity and plays a central role in the pathogenesis of allergic disorders. Despite the well established effector functions of IgE antibodies in allergy, relatively little is known about the regulation of IgE-dependent mast cell activation. IgE-dependent activation of mast cells leads to an allergic reaction within minutes if allergen exposure persists. Mast cell stimulation may result in chronic inflammation and severe tissue damage. To study the mechanisms of IgE induced mast cell activation and a potential role of inhibitory and activating Fcγ; Rs, Fel d 1 – specific monoclonal antibodies recognizing 3 different epitopes were generated and expressed as IgG1, IgG2a and IgE antibodies. These monoclonal antibodies were used to determine the number of different IgE antibodies required for mast cell activation and to reveal the mechanism of IgG-mediated inhibition of IgE-induced mast cell activation.

P18

Regulation of IgE response by targeting IgE-expressing B cells

Vogel M., Buschor P., Zwicker M., Fux M., Stadler B.M.
Universitätsinstitut für Immunologie, Inselspital, Bern, Switzerland

IgE plays a major role in the pathogenesis of allergic disease. The cross-linking of IgE-bound receptors on mast cells and basophils by multivalent allergens results in the release of mediators. Treatment using humanized anti-IgE antibodies leads to a decrease of serum IgE. Thus it has been proposed that anti-IgE can inhibit or lyse IgE-expressing B cells but clinical trials have not confirmed this hypothesis. To resolve the question whether IgE-producing B cells can be downregulated we investigate the relationship between Cε transcripts and soluble IgE protein synthesis. For that we first established an *in vitro* system for IgE synthesis by cultivating peripheral blood mononuclear cells (PBMC) from human donors with anti-CD40 antibody and IL-4 for various time points. To quantify soluble IgE synthesis and C-ε-transcripts (ε-germline, productive and membrane IgE) ELISA and real time PCR were performed, respectively. Expression of ε-transcripts appeared already after 2 days and for germline and productive IgE peaked around day 7 whereas production of soluble IgE reached maximum level at day 9. Interestingly for membrane IgE maximal transcription was already reached at day 5 suggesting that targeting B-cell expressing membrane IgE at early stage of B cell differentiation would be an approach to control IgE synthesis. Thus in order to verify this anti-IgE antibodies are currently tested and added at different time points to PBMC cultures. In parallel as it has been shown that the low affinity IgE receptor, FcεRII (CD23) acts as negative feedback for IgE synthesis we are investigating the possible interaction of B cell receptor (mIgE) with CD23 for the regulation of IgE synthesis. For that we are currently isolating CD23 novel binding molecules (not based on antibody structures) and establishing cell system assays to test their biological function *in vitro* alone or in combination with anti-IgE antibodies

P19

Comparison of LTT and CD69 measurment in drug allergy diagnosis – a prospective study

Zawodniak A., Lochmatter P., Oesch S., Kawabata T., Schmid P., Beeler A., Pichler W.J.
Clinic of Rheumatology and Clinical Immunology, Allergology, Inselspital, Bern, Switzerland

Introduction: Currently available tests to detect T cell sensitization to a drug are skin tests and in vitro lymphocyte transformation tests (LTT). Recently, upregulation of the early activation marker CD69 on T cells was found to correlate to LTT and was therefore proposed as an alternative in vitro test for diagnosis of drug allergy. CD69 measurement has the advantage that results are available already within 48 h and that no radioactivity is involved.
Aim: Comparison of both in vitro methods in a prospective study analyzing peripheral blood cells of drug allergic patients and 20 healthy, drug exposed controls.
Methods: CD69 expression was measured by flow cytometry on CD3 positive and CD3 negative cells after 48 h stimulation. For LTT, cells were stimulated 7 days, and proliferation was measured by 3H-thymidine incorporation. We performed 238 CD69 tests with 82 different drugs in 125 allergic patients. The probability of drug hypersensitivity was determined in each case based on history, skin tests and reaction upon re-exposure and classified as <10%, 10–50%, 50–90% and >90%. In the control group we performed 66 CD69 tests with 20 different drugs in 20 healthy donors who tolerated tested drugs. LTT was performed in all allergic and healthy donors.
Results: The most frequently tested drugs were antibiotics (in particular amoxicillin) and NSAIDs (107 and 34 tests in allergic patients, 26 and 29 tests in healthy donors, respectively). Drug-exposed controls were negative in both LTT and CD69 upregulation. In preliminary analysis of patients with >90% clinical probability of drug reaction (n = 20) a culprit drug could be identified in 50% of cases using CD69 measurement. Only 32% were positive using LTT.
Conclusions: These preliminary data indicate that CD69 measurement may be a useful tool and is actually more sensitive to detect T-cell mediated responses than the LTT.

P20

The TSLP receptor on cutaneous dendritic cells but not T cells is critical for the induction of TH2 contact hypersensitivity to FITC

Zimmerli S.C., Hauser C.
University Hospital of Geneva (HUG), Geneva, Switzerland

Atopic dermatitis patients exhibit upregulated thymic stromal lymphopoietin (TSLP) expression in lesional keratinocytes and forced expression of TSLP in the epidermis of mice results in an atopic dermatitis-like phenotype. We previously demonstrated that dibutylphtalate (DBP), the vehicle used to induce contact hypersensitivity (CHS) to the hapten FITC, upregulated TSLP expression in the skin. Furthermore, ear swelling and cutaneous eosinophil infiltration in response to FITC challenge as well as Th2 cytokines and serum IgE levels in sensitized mice were selectively reduced in TSLPR–/– mice. The present study was aimed at defining the relative role of the TSLP receptor (TSLPR) in CHS to FITC.
Methods: WT and TSLPR–/– mice were sensitized with 0.5% FITC/DBP. The phenotype of dendritic cells (DC) and T cells was analyzed. Finally, CFSE-labelled WT or TSLPR–/– lymph node (LN) cells were adoptively transferred into WT or TSLPR–/– recipients and FITC/DBP-induced CSFE dilution was analyzed.
Results: TSLPR–/– skin-derived but not blood derived hapten bearing DC accumulated less efficiently than WT DC in the draining (D) LN in response to FITC sensitization. DC in DLN of TSLPR–/– mice up-regulated significantly less CD86 and were less stimulatory for D11.10 T cells in the presence of ovalbumin than WT DC. Compared to WT cells T cells from TSLPR–/– cells were less activated as assessed by CD69 expression and proliferated less in vivo after FITC-sensitization as analyzed by Ki-67 expression and BrdU uptake. CSFE diluted less in TSLPR+/+ T cells in TSLPR–/– recipients than in WT recipients and TSLPR–/– T cells in WT recipients, pointing to the TSLPR on antigen-presenting cells of recipients as critical for donor T cell expansion.
Conclusion: Together, these results strongly suggest that TSLPR on cutaneous DC but not T cells plays a critical role in the induction of CHS to FITC.

P21

Evaluation of high speed laser doppler imaging technology to measure skin prick tests in daily allergy practice

Zuber J.-P., Serov A., Pavlov I., Aubert V. and Spertini F.
CHUV, Service d'Immunologie et d'Allergie, Lausanne, Switzerland

Background: Skin prick tests (SPT) are commonly used in daily allergy practice to evaluate skin reactivity to various allergens. The usual visual evaluation of SPT is observer dependent. To evaluate an objective measurement of skin reactivity to SPT, we compared the conventional visual reading (CVR) with high speed laser Doppler imaging (HS-LDI).
Methods: In a first phase, SPT with birch and grass pollen extracts (Stallergènes, France) were applied to 20 volunteers (10 atopics, 10 non atopics) to determine test characteristics (cut-off, optimal reading time and allergen concentration). Second, 30 atopic patients were tested at optimal allergen concentrations and time window with birch and grass pollen extracts. Skin reactivity was measured with CVR and HS-LDI.
Results: The optimal extract concentration was 1/1 whereas the optimal time window for the reading of SPT with HS-LDI was 5–10 min. for histamine and 10–15 min. for allergens. Taking the clinical expression of rhino-conjunctivitis as standard, the sensitivity of CVR and of HS-LDI was 96% and 100% respectively, whereas the specificity of CVR and of HS-LDI was 91% and 83% respectively.
Conclusions: Analysis of SPT with HS-LDI proved to be a simple and sensitive methodology to evaluate skin reactivity. HS-LDI test characteristics were comparable to CVR. HS-LCI could advantageously replace CVR in studies where objective measurement of skin reactivity is required.

Posters – Basic immunology

P22

Function of CXCL13 is affected by cathepsin B processing

Albrecht St., Hasan L., Heller M., Wolf M.
Theodor-Kocher Institute, Medical faculty, Bern, Switzerland

Proteolytic processing controls and fine-tunes chemokine function. Depending where the cleavage occurs, chemokines may have enhanced or reduced receptor affinity which affects their chemotactic potential. We are interested in the processing of chemokines by cathepsins and found CXCL13 to be a substrate for cathepsin B.

Processing of CXCL13 was very rapid and a stable cleavage product with an apparent molecular weight of 8675 Da was obtained. Mass spectrometry analysis pointed to a cleavage between Arg72 and Ser73 and identified CXCL13(1-72) as the cleavage product. Cleavage by cathepsin B resulted in the removal of 15 amino acids from the C-terminus, including the positively charged amino acid cluster Lys83-Arg84-Lys85, which may be crucial for binding to negatively charged glycosaminoglycans (GAGs). Chemokine-GAG interactions add to increased local chemokine concentrations and the formation of

chemokine gradients. We demonstrated that removal of 15 amino acids from the C-terminus by proteolysis reduced binding to heparin. Receptor-binding on the other hand, seems not to be affected since CXCL13(1-72) was still able to mobilize intracellular calcium and to internalize its receptor, CXCR5. The chemotactic efficacy of CXCL13 (1-72), however, was significantly changed. CXCL13 is important for B cell migration and the development of B cell follicles in secondary lymphoid organs like lymph nodes or tonsils. We are therefore investigating if chemokine cleavage by cathepsin B might occur in these organs and studied cathepsin B expression in human tonsils. Western blot experiments demonstrated that tonsil fibroblasts are a rich source of cathepsin B. Furthermore, isolated and cultivated fibroblasts secreted active cathepsin B into the culture medium as determined with the specific substrate Z-Arg-Arg-AMC.

P23

Two distinct conformations of Ly49 NK cell receptors mediate trans and cis recognition of MHC class I ligands

Back J., Scarpellino L., Schneider P., Mariuzza R.A., Held W.
Ludwig institute for cancer research, Innate immunity, Epalinges, Switzerland

Mouse NK cells express inhibitory Ly49 family receptors that bind MHC class I molecules (MHC-I) on host cells. This ensures self-tolerance of NK cells to normal host cells and allows reactivity to cells with deficient MHC-I expression. Besides interacting with ligands expressed on other cells, several Ly49 receptors also bind MHC-I ligand expressed by the NK cell itself (cis interaction). This interaction sequesters Ly49 receptors and does not induce inhibitory signaling. The molecular basis of cis versus trans interaction and reason for the distinct functional outcomes of the two types of interactions are currently not known. Here we have resolved the crystal structure of Ly49L with or without a 40-residue portion of its stalk region. This information together with the functional characterisation of a series of Ly49A variants showed that cis and trans binding of MHC-I molecules are mediated by two distinct receptor conformations. Trans binding is mediated by a conformation in which the two ligand binding domains of the homodimeric Ly49 receptor are backfolded onto the unusually long stalk region. This receptor conformation binds ligand in a bivalent fashion. In contrast, in the cis complex, the ligand binding domains of Ly49 receptor are dissociated from the stalk and therefore drastically reoriented relative to the cell membrane, allowing the binding of a single MHC-I in cis. This study provides the first structural insights for ligand binding in cis versus trans and suggests a basis for why these two interactions have distinct functional consequences. The data highlight that stalk regions can have a critical role in the function of cell surface receptors.

P24

Contribution of IL-6 and APRIL to the establishment and the maintenance of bone marrow plasma cells

Belnoue E., Tougne C., Schneider P., Huard B., Lambert P.-H, Siegrist C.-A.
University of Geneva, Pathology-Immunology, Geneva, Switzerland

The persistence of serum IgG antibodies depends first on the establishment and secondly on the long-term survival of antibody-secreting plasma cells, a complex process which essentially takes place in the bone marrow. Numerous molecules support plasmablast and plasma cell survival in vitro, the most potent factor being IL-6, but their in vivo contribution remains unclear. We used an adoptive transfer model of plasmablasts synchronized by immunization to separately assess the establishment and the persistence of plasma cells in the bone marrow compartment of gene deficient mice. This demonstrates that IL-6 is not required for the establishment of the bone marrow plasma cell pool – in contrast to APRIL. Unexpectedly, neither IL-6 nor APRIL is necessary for the persistence of plasma cells after their establishment and/or final differentiation in the bone marrow.

P25

SerpinB1 regulates the size of the neutrophil reservoir in the bone marrow independently of neutrophil elastase

Benarafa C., Lecuyer T.-E., Remold-O'Donnell E.
Immune Disease Institute, Dept of Pediatrics, Harvard Medical School, MA 02115, Boston, United States

The role of neutrophil serine proteases (NSPs), including elastase, cathepsin G and proteinase-3, in the bone marrow remains to be fully established. SerpinB1 is an endogenous inhibitor of the NSPs and we investigated here the role of SerpinB1 in neutrophil development and homeostasis. SerpinB1 was found in all human blood and bone marrow leukocytes, including stem cells. Levels of intracellular SerpinB1 were the highest in the neutrophil lineage and peaked at the promyelocyte stage, which interestingly coincides with the production and packaging of NSPs in the primary (azurophil) granules. Absolute and relative neutrophil numbers were dramatically reduced in the bone marrow of serpinB1-/- mice. Pulse-chase analysis with the thymidine analog EdU and staining with the cell cycle marker Ki-67 revealed that the defect was largely due to a loss of post-mitotic mature neutrophils within the bone marrow. Colony forming assays indicated normal numbers of myeloid progenitors. Interestingly, steady state G-CSF levels were significantly increased in serum of serpinB1-/- mice. After inflammatory challenge, the reservoir of mature bone marrow neutrophils was further depleted in serpinB1-/- mice compare to wild-type mice. Finally, we found that neutrophil elastase-deficient (ela2-/-) mice had an increased reservoir of mature neutrophils in the bone marrow but, surprisingly, double-deficient mice (ela2-/-.serpinB1-/-) had a similar phenotype as serpinB1-/- mice indicating non-overlapping pathways of regulation. Taken together these results indicate that SerpinB1 protects neutrophils from a premature death in the bone marrow and that the neutropenia is partly compensated by a regulatory feedback mechanism through increased G-CSF production. These findings demonstrate that SerpinB1 is a key regulator of neutrophil homeostasis independently of neutrophil elastase.

P26

Repetitive pertussis toxin administration protects against experimental autoimmune encephalitis

Benkhoucha M., Lalive P.-H.
CMU, Pathologie Immunologie, Genève 04, Switzerland

Objective: Pertussis toxin (PTX) in association with myelin antigens is commonly used to induce experimental autoimmune encephalitis (EAE). Known PTX effects are activation of both T cells and antigen-presenting cells and permeabilization of the blood-brain barrier (BBB). We addressed the question whether continuous PTX pre-treatment could alter the course of MOG-induced EAE.
Methods: C57BL/6 mice were injected weekly over 6 months with 300 ng PTX iv. EAE was induced in PTX pre-treated (PT; n = 8) and non-PTX pre-treated (NPT; n = 10) with MOG35-55.
Results: Before EAE induction, T cell proliferation to specific (PTX) and unspecific (PHA) stimuli was similar in both groups, excluding any tolerization effect. After immunization, EAE was significantly delayed and ameliorated in the PT group compared to NPT group. At a progressed EAE stage, T cell proliferation following PTX stimulation was not different between groups. In contrast, PT mice showed a significantly reduced T cell proliferation and IFNγ production in response to MOG35-55 stimulation. When PTX was used as recall Ag, a strong IL-10 induction was found in the splenic population of the PT group. Furthermore, we observed by FACS a strong PTX-inducible expansion of CD4+-CD25+-Foxp3+cells in the PT group before EAE induction.

P27

Dissecting the role of ICAM-1 and ICAM-2 during lymphocyte recirculation

Boscacci R., Pfeiffer F., Gollmer K., Deutsch U., Engelhardt B., Stein J.-V.
University of Bern, Theodor Kocher Institute, Bern Switzerland

Lymphocytes continuously recirculate through secondary lymphoid organs such as peripheral lymph nodes (PLN) in search of their specific antigen. LFA-1 interaction with endothelial ICAM-1 and ICAM-2 is involved in lymphocyte recruitment across high endothelial venules (HEV), specialized postcapillary venules of PLN. However, their relative contribution to lymphocyte arrest on the HEV versus lymphocyte diapedesis across the HEVs and interstitial migration has not been addressed. Performing intravital microscopy studies we show, that genetic ablation or blocking of ICAM-1 reduced the firm adhesion of lymphocytes in HEVs by approximately 50% when compared to control mice. Functional absence of ICAM-2 alone did not affect firm adhesion, although we observed a further reduction of lymphocyte adhesion in HEV, when ICAM-1 was absent, suggesting that both ICAM-1 and ICAM-2 contribute to lymphocyte adhesion within HEV. Performing short term in vivo homing studies using "quantitative 3D-immunohistology", we found that functional absence of endothelial ICAM-1 and -2 led to an 80 % reduction in lymphocyte homing to PLN after cell transfer, with residual homing dependent on endothelial VCAM-1. Of note, accumulation of transferred cells within lymphoid tissue was only midly impaired in ICAM-1/-2 double deficient PLN. This suggested that transendothelial migration could occur in absence of endothelial ICAMs. Finally we investigated the requirement for ICAM-1 during interstitial lymphocyte migration, using two photon microscopy (2PM). B cells motility was slightly reduced in absence of ICMA-1, although robust migration was still observed. In summary our analysis suggests a central role for ICAM-1 and -2 during shear-resistant adhesion of blood-borne lymphocytes in HEV. Transmigration through HEV and interstitial migration, on the other hand, did not absolutely require ICAM-1 and -2.

P28

Insight into mechanism of IL-2-induced pulmonary edema provides rationale for improved IL-2 immunotherapy

Boyman O., Krieg C., Létourneau S., Pantaleo G.
University Hospital of Lausanne (CHUV), Division of Immunology and Allergy, Lausanne, Switzerland

IL-2 immunotherapy is used against certain metastatic cancers and for patients infected with human immunodeficiency virus. However, administration of IL-2 can lead to serious side effects including pulmonary edema, the pathogenesis of which is ill-defined. We will provide data showing that IL-2 may bind directly to CD25 on lung endothelial cells and thus cause toxicity and pulmonary edema. Thus, lung endothelial cells express CD25 under steady-state conditions, and expression levels of CD25 on these cells increase in vivo upon injection of mice with IL-2. Activation of CD25+ lung endothelial cells and of IL-2 receptor-positive NK and T cells leads to pulmonary edema upon IL-2 treatment. Significantly, co-administration of anti-CD25 antibody along with IL-2 treatment is able to completely abrogate IL-2-induced pulmonary edema despite considerable proliferation of NK and T cells. Similarly, knocking out CD25 on non-immune cells or obscuring the CD25-binding epitope of IL-2 by the use of selective immune complexes of IL-2 plus anti-IL-2 antibody (IL-2/mAb) is able to prevent IL-2-induced pulmonary edema. Notably, IL-2-mediated toxic effects are also evident on a human lung cell line and can be abrogated using the anti-CD25 antibody daclizumab or IL-2/mAb complexes. Moreover, IL-2/mAb complexes are very efficient in generating anti-tumor responses in vivo against syngeneic local or metastatic tumor nodules. Thus, CD25+ endothelial cells play a crucial role in IL-2-mediated vascular leak syndrome and this can be circumvented by the use of IL-2/mAb complexes.

P29

Characterization of a novel Melan-A/Mart-1 CD4 T cell epitope in a vaccinated melanoma patient developing a tumour-specific CTL response

Braun M., Jandus C., Baumgartner P., Schwarz K., Bachmann M., Speiser D., Romero P.
Division of Clinical Onco-Immunology, Ludwig Institute for Cancer Research, Lausanne

Current immunotherapy protocols focus on the induction of high numbers of functional cytolytic T lymphocytes (CTLs) that efficiently target the tumour. The generation of a long-lasting tumour-specific CTL response is CD4 T cell help-dependent for many antigens. Here, we assessed the specific CD4 T cell response in HLA-A2 melanoma patients vaccinated with a VLP linked Melan-A peptide containing the immunodominant Melan-A_{26-35} epitope. One out of nine patients showed a strong CD4 T cell response. A vaccine-specific CD4 T cell line and 10 clones were generated. Anti-HLA class II antibodies and a panel of EBV-B cell lines lead to determine that the Melan-A specific CD4 T cells were HLA-DQB1*0202 restricted. The minimal epitope was mapped using short overlapping peptides with progressive N- or C-terminal truncations. The minimal epitope recognized by the polyclonal T cell line was defined by the peptide Melan-A_{18-28}. Initial results from tumour recognition assays suggest that the antigen may be naturally processed. Since this responding patient showed the highest frequency in HLA-A2/Melan-A multimer+ CD8 T cells after the final vaccine boost, with a high increase in central memory phenotype, it is tempting to hypothesize that the concurrent generation of Melan-A antigen specific CD4 T cells may have facilitated the generation of a robust tumour antigen specific memory CD8 T cell response. These results may support the use of synthetic peptides bearing both class I and class II epitopes as promising vaccine candidates and emphasize the importance of inclusion of strong CD4 T cell epitopes in peptide vaccination strategies.

P30

Role of costimulatory molecules in LatY136F CD4 T cell mediated B cell activation

Chevrier S., Genton C., Malissen B., Malissen M., Acha-Orbea H.
University of Lausanne, Dpt of Biochemistry, Epalinges, Switzerland

LatY136F mutant mice present a point mutation in the LAT adaptor protein involved in T cell receptor signaling. As a consequence of this mutation very few T cells develop in the thymus but the few CD4 T cells which reach the periphery undergo a strong IL-7 dependent homeostatic expansion. These expanding CD4 T cells are TH2 polarized and induce a strong polyclonal B cell activation leading to an autoimmune disorder characterized by glomerulo-nephritis with IgE deposits and proteinuria. Since this B cell activation occurs in an MHC II independent manner, we sought to understand the molecular mechanisms responsible to trigger this B cell activation. In vitro co-culture of LatY136F CD4 T cells and B cells deficient for costimulatory molecules allows us confirming that MHC II is not involved in that process and demonstrating that CD80 and CD86 as well as IL-4 and LFA-1 are required to induce IgG1 secretion. On the contrary, CD40 and IcosL signaling were not necessary for the in vitro activation of B cells. An adoptive transfer system was used to analyze the effect of CD40, CD80/CD86 and IcosL in vivo. We showed that CD80/CD86 were required for initial T cell expansion whereas CD40 and IcosL deficiency led to a less efficient B cell response.

P31

On the role of monoclonal antibody affinity in mediating protection against autoimmune inflammatory diseases

Dallenbach K., Röhn T., Beerli R., Bachmann M.F.
Cytos Biotechnology AG, Schlieren, Switzerland

Monoclonal antibodies (mAbs) have recently emerged as new drug modalities for the treatment of chronic inflammation. Indeed, blocking cytokines, such as TNF, using mAbs has been established as disease-modifying therapy for inflammatory diseases including rheumatoid arthritis and psoriasis. It is generally assumed that mAbs need to have a high affinity for the target cytokine in order to show efficacy. However, no study has ever directly addressed this issue. To elucidate this question, we have generated a panel of IL-17-specific antibodies. In order to vary the affinity of the mAbs, we will pick high-affinity antibodies and mutate their variable regions towards germ-line, which is expected to lower their affinity for IL-17. The ability of these antibodies, which recognize the same epitope with different affinities, to block chronic inflammation will subsequently be tested in two murine models of autoimmunity: collagen-induced arthritis and EAE. To estimate the importance of antibody-induced immune complex formation for removal of the cytokine from the circulation, cocktails of antibodies recognizing different epitopes on IL-17 will be compared to single antibody treatment. These experiments will reveal the role of antibody affinity versus their ability to induce immune complex formation for the treatment of autoimmune diseases.

P32

Ticks produce chemokine binding proteins with stringent and broad specificities

Déruaz M., Power C., Proudfoot A.E.I.
Merck Serono Research Centre, Immunology, Geneva, Switzerland

The expression of chemokine-binding proteins is a known strategy used by parasites such as viruses to suppress the infiltration of lymphocytes to the infection site allowing them to evade from the host immune system. Blood-feeding parasites such as ticks have been shown to express cytokine and chemokine-inhibiting factors. We have investigated the chemokine-binding activity of saliva of the Rhipicephalus sanguineus tick and first identified two highly selective proteins: Evasin-1, which inhibits CCL3/MIP-1α, CCL4/MIP-β and CCL18/PARC and Evasin-3, which inhibits CXCL8/IL-8 and CXCL1/Gro α. More recently we identified a third chemokine-binding protein: Evasin-4. Contrarily to Evasin-1 and -3, Evasin-4 shows a broader specificity and inhibits 12 different CC-chemokines as measured by surface plasmon resonance, receptor binding and chemotaxis assays. All three proteins have anti-inflammatory activity in disease models. Evasin-1 and Evasin-4 show conserved Cys residues, implicating that they will have a similar fold, whereas Evasin-3 has no sequence homology. The three dimensional structures of Evasin-1 and Evasin-3 display novel folds, and bear no resemblance to each other.

P33

PMN-Ect modulate the inflammatory response of macrophages via MerTK regulated PI3K/Akt and NFκB pathways

Eken C., Martin P.-J., Sadallah S., Treves S., Schaller M., Schifferli J.-A.
University Hospital Basel, Biomedicine Department - Immunonephrology Lab., Basel, Switzerland

At the earliest stage of activation, human polymorphonuclear neutrophils (PMNs) release vesicles, which bud from the cell surface. These vesicles, called ectosomes (PMN-Ect), expose phosphatidylserine in the outer membrane leaflet. They inhibit the inflammatory response of human monocyte-derived macrophages and dendritic cells to LPS and zymosan, and induce TGFβ1 release, suggesting a reprogramming towards a tolerogenic phenotype. The receptors and signaling pathways involved have not yet been defined. PMN-Ect containing supernatants of fMLP-stimulated PMNs from healthy donors were concentrated by ultracentrifugation. PMN-Ect induced an immediate calcium flux in macrophages indicating that signaling processes were immediately activated. PMN-Ect interfered with zymosan A activation of macrophages via inhibition of NFκB translocation and NFκB p65/RelA phosphorylation. Mer tyrosine kinase receptor (MerTK) and phosphatidylinositol 3-kinase (PI3K)/Akt pathway played a key role in this immunomodulatory effect as shown by using specific MerTK blocking antibodies and LY 294002, a PI3K inhibitor. As a result, PMN-Ect reduced the transcription of many pro-inflammatory genes in zymosan A activated macrophages. Of interest, the TGFβ1 release induced by PMN-Ect was not related to a

modification in its transcription. In sum, PMN-Ect modulated the inflammatory response of macrophages by different means including rapid signaling possibly responsible for the release of TGFβ1, and via MerTK regulated PI3K/Akt and NFκB pathways.

P34

Selective requirement for C-MYC at an early stage of Vα14i NKT cell development

Mycko M. P., Ferrero I., Wilson A., Jiang W., Bianchi T., Trumpp A., MacDonald H. R.
Ludwig Institute for Cancer Research, Lausanne Branch, University of Lausanne Ltd., Epalinges, Switzerland

Vα14 invariant (Vα14i) natural killer T (NKT) cells are a subset of regulatory T cells that utilize a semi-invariant TCR to recognize glycolipids associated with monomorphic CD1d molecules. During development in the thymus $CD4^+CD8^+$ Vα14i NKT precursors recognizing endogenous CD1d-associated glycolipids on other $CD4^+CD8^+$ thymocytes are selected to undergo a maturation program involving sequential expression of CD44 and NK-related markers such as NK1.1. The molecular requirements for Vα14i NKT cell maturation, particularly at early developmental stages, remain poorly understood. Here we show that CD4-Cre mediated T cell-specific inactivation of c-Myc, a broadly expressed transcription factor with a wide range of biological activities, selectively impairs Vα14i NKT cell development without perturbing the development of conventional T cells. In the absence of c-Myc Vα14i NKT cell precursors are blocked at an immature $CD44^{low}NK1.1^-$ stage in a cell autonomous fashion. Residual c-Myc deficient immature Vα14i NKT cells appear to proliferate normally, cannot be rescued by transgenic expression of BCL-2 and exhibit characteristic features of immature Vα14i NKT cells as high levels of PLZF and IL4 pre-formed RNA. Collectively our data identify c-Myc as a critical transcription factor that selectively acts early in Vα14i NKT cell development to promote progression beyond the $CD44^{low}NK1.1^-$ precursor stage.

P35

IL-1α expression and secretion

Fettelschoss A., Weiss A., Kündig T.M.
University Hospital Zurich, Dermatology, Zurich, Switzerland

IL-1α and -β are important proinflammatory cytokines. In contrast to IL-1β, IL-1α can be expressed on the cell surface and does not require cleavage to be biologically active. However, the exact mechanisms of IL-1α cell surface expression and of IL-1α secretion are unknown. We are investigating which factors influence and regulate IL-1α surface expression and secretion using an in vitro model involving LPS stimulated monocytes and macrophages. At different time points the amount of intracellular IL-1α, surface IL-1α, and IL-1α secretion are measured by FACS analysis, and ELISA, respectively. IL-1α is expressed in various tumor types, and has been shown to correlate with tumor progression. Therefore we are evaluating the relative biological importance of the different IL-1α forms in tumor cells, i.e. the intracellular, the cell surface, and the secreted form. We have generated different EL4 cell lines expressing either intracellular IL-1α, surface IL-1α, or only secreted IL-1α. These different tumor cell lines shall be compared in tumor mouse models using either C57BL/6 or IL-1RI–/– mice. We will quantify tumor growth, angiogenesis and metastasis formation.

P36

TSLP can substitute for IL-7 in lymph node development

Finke D., Chappaz S.
Department of Biomedicine, Developmental Immunology, Basel, Switzerland

Lymph node (LN) organogenesis is initiated by the interaction between the hematopoietic lymphoid tissue inducer (LTi) cells and mesenchymal organizer cells. Mice in which the interleukin (IL)-7 signaling pathway has been disrupted have a severe defect in LN development, but the reasons underlying this defect are unknown. Here, we show that overexpression of thymic stromal lymphopoietin (TSLP) restored LN development and increased LTi cell numbers in IL-7-/- and RAG2-/- γc-/- mice. Chimera experiments revealed that TSLP promoted the generation of LTi cells from fetal liver precursors. TSLP-mediated LN restoration was strictly dependent on LTi cells and occurred independently of lymphocyte colonization. Increased LTi cell numbers in the LN anlagen of RAG2-/- γc-/- TSLP transgenic mice were associated with the restoration of organizer cells. These results show that lymphocyte colonization is not required for persistence of the LN anlage and suggest that the LN defect in mice deficient for molecules of the IL-7 signaling pathway is the consequence of insufficient LTi cell numbers. Our results identify LTi and organizer cells as the minimal cellular players required for LN organogenesis and demonstrate that TSLP can substitute for IL-7 in this process.

P37

Thymic crosstalk regulates Delta-like 4 expression on cortical epithelial cells

Fiorini E., Ferrero I., Merck E., Favre S., Pierres M., Luther S. A., MacDonald H. R.
Ludwig Institute for Cancer Research, Lausanne Branch, University of Lausanne, Epalinges, Switzerland

Interactions between Notch1 (N1) receptors on lymphoid progenitors and Delta-like4 (DL4) ligands on thymic cortical epithelial cells (cTEC) are essential for T cell lineage commitment, expansion and maturation in the thymus. Using a novel mAb against DL4 we show here that DL4 levels on cTEC are very high in the fetal and neonatal thymus when thymocyte expansion is maximal, but decrease dramatically in the adult when steady state homeostasis is attained. Analysis of mutant mouse strains where thymocyte development is blocked at different stages indicates that lymphostromal interactions ("thymus crosstalk") are required for DL4 downregulation on cTEC. Reconstitution of thymocyte development in these mutant mice further suggests that maturation of thymocytes to the $CD4^+$ $CD8^+$ stage and concomitant expansion is needed to promote DL4 downregulation on cTEC. Collectively our data support a model where thymic crosstalk quantitatively regulates the rate of N1-dependent thymopoiesis by controlling DL4 expression levels on cTEC.

P38

Purinergic signalling in T cell development

Frascoli M.
Institute for Research in Biomedicine, T cell development, Bellinzona, Switzerland

Plasma membrane receptors for extracellular nucleotides termed P2 receptors, which are divided in two families, P2X and P2Y, are found on almost all cell types. P2X1 to P2X7 receptors are permeable to monovalent cations and some P2X receptors exhibit substantial permeability to Ca2+ or anions, whereas P2Y receptors are guanine nucleotide-binding protein (G protein)-coupled receptors (GPCRs), which bind preferentially to adenosine diphosphate (ADP), uridine diphosphate (UDP), uridine triphosphate (UTP), or UDP-glucose. In thymocytes, ATP might control cell growth and proliferation through P2 receptors. Analysis of transcriptional regulation of purinergic receptors in thymocyte subsets by real time (RT) PCR revealed low expression of P2X4, P2X7 and P2Y14 receptors in the precursors of the ab lineage, whereas the same receptors were abundantly expressed in gd cells. To see whether P2X signalling was involved in pre-TCR/Notch signalling we treated E14 fetal thymus organ cultures (FTOC) with oxidized ATP (oATP), a P2X receptor antagonist. We observed a dramatic reduction of cellularity with selective inhibition of the transition of TCRβ expressing DN3 thymocyte to the DN4 and DP stage by oATP. The same inhibition was observed in co-culture of DN3 thymocytes with OP9-DL1 cells. These results suggest a crucial role of purinergic signalling at the β selection checkpoint. Lineage choice in immature T cells is dictated by TCR signal strength with increasing signalling resulting in gd T cell development. Artificial reduction of gdTCR signalling was shown to divert gdTCR expressing cells toward the ab fate. If pericellular ATP were controlling signal strength as observed in mature T cells, then gdTCR bearing thymocytes would be diverted to the ab lineage in the presence of oATP. Indeed, we observed generation of CD4+8+ cells (e.g. ab committed) expressing gdTCR in FTOC treated with oATP. Our results indicate that ATP influences T cell lineage choice during thymic development. We are investigating the source of ATP in the organ microenvironment and whether ATP concentration impacts on functional potential of developing T cells.

P39

Gut lymphocytes selectively express a TH17 or a regulatory phenotype and specific activation patterns according to their localization and to TCRγδ or TCRαβ expression

Frossard C.-P., Asigbetse K.-E., Eigenmann P.-A.
Hôpital Universitaire de Genève, Pédiatrie, Genève, Switzerland

In mice, T cell localized in the small intestine represent almost 50% of the total T cells number. The gut is a specific physical barrier, separating a milieu loaded with microbial and food antigens, and the sterile environment of the submucosa. Constant antigen stimulation modifies the T cell population phenotypes during lifetime. The aim of the present study was to characterize, by flow cytometry, the phenotypes and activation patterns of T lymphocytes isolated from various localizations of the gut (intra-epithelia lymphocytes (IEL), lamina propria lymphocytes (LPL), Peyer patches and mesenteric lymph nodes) and systemic compartments (blood and spleen) in young naïve mice and animal orally activated with cholera toxin. We focus in particular on T cell receptor (TcR) αβ and TcRγδ expression as well as activation markers (CD25, CD44, CD45RB, CD69), homing molecules, (CCR9 and aIELb7) and the cytokine profile after in vitro and in vivo activation (IFNγ, IL-4, IL-10 and IL-17) and FoxP3 expression.

The analysis of the phenotypes of T cells isolated from various locations of the gut, as well as the systemic compartment has shown TcRαβ CD4+ or CD8αβ+ are preferentially found in the systemic compartment whereas IEL and LPL are characterized by a predominance of TCRγδ+/CD8 αα+ T cells. T cells from IEL and LPL express specifically the activation markers CD44, CD69 and CCR9, in contrast TcRαβ express a phenotype of naives T lymphocyte. In addition IFNγ and IL-4 were not detected on T cells populations after 4h in vitro activation with PMA-ionomycine or after in vitro activation with cholera toxin. Interestingly, in vivo and in vitro activation induce rapidly (4 h) by LPL TcRγδ+/CD8αα+ the secretion of IL-10 and IL-17, suggesting a Th17 phenotype. In addition, 20% of TcRαβ+/CD4+ LPL express FoxP3, suggesting a regulatory function.

P40

Engineering a functional thymus from adult thymic epithelial cells

Gill J., Kenins L., Zuklys S., Holländer G.
University of Basel, Department of Biomedizin, Basel, Switzerland

The thymus is the primary lymphoid organ responsible for the lifelong generation of T lymphocytes. The ability of the thymus to support T lymphocyte development is intimately linked to specialized functions and architecture of thymic stromal cells, which are mainly comprised of diverse subsets of thymic epithelial cells (TECs). TECs control the homing, expansion, maturation and selection of developing T lymphocytes and are indispensable for the formation and maintenance of the acquired immune system. While functional and developmental defects of the thymus severely compromise the adaptive immune system and can cause life-threatening immunodeficiency or autoimmunity, a limited understanding of the cellular and molecular mediators of TEC development and function have hindered tissue engineering of the thymus, to correct such debilitating defects. TEC replacement therapy using adult derived cells has been unsuccessful because of the inability to identify tissue-specific precursors that can be maintained and expanded while preserving their competence to support T cell development. Here we demonstrate that the transient, ectopic expression of Oct4 in TECs from adult mice reveal a previously unrecognized potential for long term in vitro maintenance and propagation of a TEC precursor population. The progeny of a single cell expanded in vitro maintains TEC functions and, upon transfer into athymic mice, gives rise to a microenvironment that supports T cell development. These data identify in adult mice a TEC population that is responsive to Oct4 activity and can be individually engineered to replace defective thymus tissue. Recent insights and new research models have led to advances in understanding of both the origin and lineage relationships of TECs. Together with identification of key genetic programs functional in thymus development and maintenance, the stage is set for elucidating mechanisms, which may allow control of TEC differentiation and function and support the evolution of improved approaches for clinical management of immune disorders.

P41

DNA-array analysis of a murine model for histiocytosis

Grosjean F., Otten L., Steiner Q., Acha-Orbea H.
Université de Lausanne, Biochimie, Epalinges, Switzerland

A transgenic murine model expressing the simian virus 40 (SV40) T antigen under the control of the CD11c promoter results in animals with massive proliferation of CD8α positive dendritic cells. These mice show transformed dendritic cells infiltrations of spleen, liver, bone marrow, thymus and different lymph nodes with similarities to multi-systemic Langerhans cell histiocytosis. Early transformation occurs in both CD8α negative and CD8α positive dendritic cells subset but is rapidly overcome by a massive CD8α positive proliferation. This results in a complete disappearance of the CD8α negative dendritic cells, as shown by flow cytometry analysis, in animals in advanced transformation stages. Wild type and transgenic, CD8α negative and CD8α positive dendritic cells population were sorted at different stages of transformation (when available) for a complete DNA-array analysis using 430_2 Affymetrix chips (containing ~45'000 probe sets per chip). While the proliferative dendritic cell subset keeps the phenotype, function and maturation capacity of wild-type dendritic cells, DNA-array analysis showed clear perturbations in cell cycle controls and proliferation between wild-type and transgenic, CD8α negative and CD8α positive dendritic cells populations. These subtype-dependant transformation differences may provide an explanation for the proliferation advantage of the CD8α positive subset.

P42

The role of GM-CSF in the induction of EAE

Hesske L., Cretton C., Reith W., Fontana A., Suter T.
University Hospital Zurich, Clinical Immunology, Zurich, Switzerland

The growth factor granulocyte-macrophage colony-stimulating factor (GM-CSF) is a well known cytokine affecting the differentiation of dendritic cells (DCs). DCs are the most important antigen presenting cells (APCs). They play a crucial role in initiating beneficial immune responses and in the development of autoimmune diseases like multiple sclerosis (MS) or its animal model experimental autoimmune encephalomyelitis (EAE). Thus, we aimed at defining the role of GM-CSF in the recruitment of different DC subpopulation and its impact on the EAE course. The RNA and protein levels of GM-CSF from spinal cord samples were analyzed during EAE development and were found to peak during the exacerbation phase of EAE, indicating a potential role of the growth factor on disease induction. In order to define the role of GM-CSF in recruiting distinct DC subsets into the central nervous system (CNS) we injected GM-CSF-producing cells intracerebrally into naive animals. This treatment resulted in an increase of total DC numbers, including myeloid CD11b+ CD4-, CD11b+ CD4+ and plasmacytoid Gr1+ B220+ DCs. Furthermore, the GM-CSF treatment of myelin oligodendrocyte glycoprotein (MOG) immunized mice resulted in a more severe EAE course. Finally, GM-CSF receptor-deficient mice did not develop EAE and did not contain any infiltrates within the CNS during disease development. In summary, the data suggest that distinct DC subpopulations are recruited into the CNS by intracerebral GM-CSF. In EAE, both the DC recruitment and the disease severity are enhanced in the presence of GM-CSF. In addition, the results with the GM-CSF receptor-deficient mice indicate that GM-CSF plays a crucial role in the induction of EAE.

P43

A safe and recombinant, tetravalent dengue vaccine induces very high antibody titers that neutralise all serotypes

Huber A.
Immunodrugs, Schlieren, Switzerland

There are 50–100 million cases of dengue fever, 500'000 cases of dengue hemorrhagic fever and dengue shock syndrome, and more than 20'000 death each year. Since there is neither a specific treatment nor a vaccine available, the only method to control dengue is to combat the mosquito transmitting the virus. A promising vaccine should induce neutralising antibodies against all the four closely related dengue serotypes (DEN1 to DEN4). Dengue viruses display on their surface an envelope glycoprotein "E" that has been the main target for neutralising antibodies and mediates viral attachment. Domain III of the envelope protein (ED3) confers the receptor binding activity and has been suggested to contain most neutralising epitopes. Now, ED3s from the four serotypes were expressed in E. coli, refolded from inclusion bodies and chemically coupled to virus-like particles (VLP). These VLPs are highly immunogenic. Four monovalent vaccines were mixed and mice immunised. Tetravalent sera from these mice had titers between 300'000 to 500'000 against each single ED3. The same sera were tested in an in vitro neutralisation assay (PRNT50) with four dengue wild type strains and a dengue susceptible cell line. The tetravalent sera neutralised strains from three different serotypes with titers between 1'000 to 6'000, and only the DEN4 strain was neutralised at a lower titer. No negative control serum did neutralise dengue in that assay. In summary, unlike the attenuated vaccines that are now in clinical trials, our vaccine is safe and induces very high antibody titers. Serum from immunized mice neutralises all the four dengue serotypes.

P44

IL-17 promotes the expansion of IL-17-producing CD4+ T cells by monocyte activation

Huber X.S., Droeser R.A., Feder-Mengus C., Oertli D., Heberer M., Spagnoli G.C., Iezzi G.
ICFS, Surgery, Basel, Switzerland

IL-17 producing CD4+ T cells (Th17) are involved in the pathogenesis of autoimmune diseases and host defence. IL-1β and IL-23 are of major importance for polarization and/or expansion of human Th17. Here we addressed the role of IL-17 itself in the elicitation of these functions. Expression of IL-17 receptor was tested by flow cytometry. CD14+ monocytes isolated by magnetic beads were stimulated with recombinant IL-17 and/or lipopolysaccharide (LPS) and expression of surface markers and cytokine genes or cytokine secretion was evaluated by flow-cytometry and ELISA. Th17 were enumerated by intracellular staining. IL-17 receptor expression in lymphocytes was negligible on CD4+ T cells and limited to a subset of <20% of CD8+ T cells. In contrast, virtually all CD14+ monocytes expressed IL-17 receptor. Culture in the presence of GM-CSF and IL-4 or IFNγ, promoting generation of dendritic cells (DC) led to complete disappearance of IL-17 receptor. These data posed the question of functional effects of IL-17 on monocytes. Incubation of CD14+ monocytes in the presence of 10–200 ng/ml IL-17 did not modulate the expression of HLA-class I or II determinants, CD80, CD83 or CD86. However, in the presence of 50–200 ng/ml concentrations of IL-17, secretion of IL-1β by monocytes stimulated with LPS (1–1000 ng/ml) significantly (2X) increased as compared to cultures performed in the absence of IL-17. Most importantly, stimulation of CD4+ T cells with

allogenic monocytes pre-incubated in the presence of LPS (1 µg/ml) and IL-17 (100 ng/ml) promoted the expansion of a number of Th17 significantly higher as compared to cultures performed with LPS alone. In particular, significantly higher percentages of memory CD4+/CD45RA- cells producing IL-17 alone or together with IFNγ were detectable (19 ± 0.19 vs. 12.2 ± 0.87, p = 0.003) in these conditions. Taken together, our data indicate that IL-17 promotes the expansion of Th17. These effects are mediated by monocyte activation.

P45

Autoantigen-specific interactions with CD4+ thymocytes control mature medullary thymic epithelial cell cellularity

Irla M., Hugues S., Gill J., Nitta T., Hikosaka Y., Williams I.R., Hubert F.X., Scott H.S., Takahama Y., Holländer G.A., Reith W.
University of Geneva, Faculty of Medicine, Geneva, Switzerland

Medullary thymic epithelial cells (mTECs) are specialized for inducing central immunological tolerance to self-antigens. To accomplish this, mTECs must adopt a mature phenotype characterized by expression of the autoimmune regulator Aire, which activates the transcription of numerous genes encoding tissue-restricted self-antigens. The mechanisms that control mature Aire+ mTEC development in the postnatal thymus remain poorly understood. We demonstrate here that, although either CD4+ or CD8+ thymocytes are sufficient to sustain formation of a well-defined medulla, expansion of the mature mTEC population requires autoantigen-specific interactions between positively selected CD4+ thymocytes bearing autoreactive T cell receptor (TCR) and mTECs displaying cognate self-peptide-MHC class II complexes. These interactions also involve the engagement of CD40 on mTECs by CD40L induced on the positively selected CD4+ thymocytes. This antigen-specific TCR-MHC class II-mediated crosstalk between CD4+ thymocytes and mTECs defines a unique checkpoint in thymic stromal development that is pivotal for generating a mature mTEC population competent for ensuring central T cell tolerance.

P46

Selected TLR ligands and viruses promote helper-independent CTL priming by upregulating CD40L (CD154) on dendritic cells

Johnson S., Zhan Y., Mount A., Belz G., Lew A.
Université de Genève, Pathology and Immunology, Geneva, Switzerland

CD40L on CD4+ T cells has been shown to license dendritic cells (DC) via CD40 to prime CTL responses. Surprisingly, we found that the converse (CD40L on DC) was also important. Anti-CD40L treatment decreases endogenous CTL responses to both OVA and influenza infection even in the absence of CD4+ T cells. DC express CD40L upon stimulation with agonists to TLR 3 and 9. Moreover, influenza infection, which stimulates CTL without help upregulates CD40L on DC, but herpes simplex infection, which elicits CTL through help, does not. CD40L–/– DC are suboptimal both in vivo in bone marrow chimera experiments and in vitro in mixed lymphocyte reactions. In contrast, CD40L–/– CD8+ T cells kill as effectively as wildtype. We conclude that CD40L upregulation on DC promotes optimal priming of CD8+ T cells without CD4+ T cells, providing a mechanism by which pathogens may elicit helper-independent CTL immunity.

P47

Adult-like neonatal protective anti-mycobacterial T-cell responses through in vivo activation of vaccine-targeted dendritic cells

Kamath A.T., Rochat A., Valenti M.P., Agger E.M., Christensen D., Andersen P., Lambert P.H., Siegrist C.A.
Department of Pathology and Immunology, University of Geneva, Geneva, Switzerland

Tuberculosis continues to be a major cause of global morbidity and mortality. As new vaccine candidates are identified, which overcome the limitations of the early life immunity, understanding the underlying interaction with the immune system is important to develop optimal formulations. Vaccine efficacy during early life largely depends upon DC targeting and activation. The most potent TLR soluble ligands induce diffuse DC activation, which may be associated with marked proinflammatory responses and possibly adverse effects. This raises the concern that effective vaccine adjuvants may similarly rely on widespread DC activation. A promising candidate vaccine against tuberculosis (Ag85B-ESAT-6) formulated in the potent cationic liposome, CAF01 induced an adult-like multifunctional IFNγ/TNFα/IL-2 response following immunization in early life, and was used to study in vivo DC targeting and activation: following the fate of antigen and adjuvant in the draining lymph nodes to define the magnitude of DC targeting/activation required in vivo to induce protective vaccine responses. Unexpectedly, protective Ag85B-ESAT-6/CAF01-induced responses were associated with the activation of a minute population of CD11c+ LN DCs, without detectable systemic proinflammatory responses. This activated peripheral tissue-derived DC population, characterized by enhanced CD80, CD86 and CD40 was only identified when focusing on adjuvant- or antigen-labeled CD11c+ DCs. Thus, potent protective multi-cytokine producing responses may be elicited by the exquisite activation of a minute number of in vivo targeted DCs in early life.

P48

Pattern recognition versus inflammation in CD8+ T cell priming by dendritic cells

Kratky W., Oxenius A., Spörri R.
ETH, Inst. f. Mikrobiologie, Zürich, Switzerland

Efficient priming of naïve T cells by dendritic cells (DCs) requires three signals: 1. The cognate peptide: MHC, 2. costimulation, and 3. immunomodulatory cytokines/etc. Only fully activated DCs which have sensed conserved microbial structures (PAMPs) via pattern recognition receptors (such as toll-like receptors) can provide all these signals. However, DCs can also be matured in trans by inflammatory mediators secreted by PAMP-triggered cells. Although DCs which were activated by this indirect mode deliver signal 1 & 2, they fail to secrete immunomodulatory cytokines such as IL-12. Consequently, indirectly-activated DCs do not promote the differentiation of naive CD4+ T cells into fully functional T helper cells, even in the presence of fully activated, but non-presenting DCs. The aim of this project is to investigate the consequences of DC activation by inflammatory mediators for subsequent T cell priming. In particular, we want to test whether the findings from experiments with CD4+ T cells also apply for CD8+ T cells. In in vivo and in vitro experiments, we show that inflammatory mediators are sufficient for DCs to upregulate molecules which are crucial for migration and subsequent CD8+ T cell priming. However, in sharp contrast to directly-activated (PAMP- triggered) DCs, indirectly-activated DCs fail to produce proinflammatory cytokines, a hallmark of full DC activation. In preliminary studies we show that these differences in DC activation seem to have major impact on CD8 T cell priming. Indirectly- activated DCs prime CD8+ T cells with defects in cytokine production and effector function. In ongoing studies we aim to study the differences between direct and indirect DC activation in more detail. We are particularly interested in aspects involved in positive and negative regulation of T cell function which may explain the differential outcome on the level of CD8+ T cells.

P49

Biological role of myeloid cell-derived interleukin-1 receptor antagonist in collagen-induced arthritis

Lamacchia C., Palmer G., Talabot-Ayer D., Gabay C.
University of Geneva School of Medicine (CMU), Division of Rheumatology and Department of Pathology, Geneva, Switzerland

The balance between interleukin-1 (IL-1) and its specific inhibitor, the IL-1 receptor antagonist (IL-1Ra) plays a major role in the development of arthritis and in the local inflammatory events leading to joint damage. In lymphoid organs, dendritic cells-derived IL-1 is required for antigen-specific T cell activation and it has been demonstrated that IL-1Ra is involved in the control of the T helper (Th17) response in arthritis. However, the contribution of myeloid cells as cellular sources of IL-1Ra in lymphoid organs has not been formally examined. In addition, activated myeloid cells, including macrophages and neutrophils are considered actually as major source of IL-1Ra in the inflamed joints.
Objective: The aim of this study was to define the relative role of myeloid cells as compared to other sources of IL-1Ra in collagen-induced arthritis (CIA).
Methods: Conditional myeloid cell-specific IL-1Ra deficient mice (IL-1RaDM) were generated in the DBA/1 background by using the LoxP/Cre-recombinase system. CIA was induced in IL-1RaDM mice and control littermates by one single immunization with bovine type II collagen (CII) in complete Freund's adjuvant. Arthritis severity was assessed by clinical scoring. Histological analysis of the joints at the end of the experiment (day 25) was used to confirm the clinical assessments of arthritis. Cytokines levels were quantified by ELISA.
Results: IL-1RaDM mice exhibited early disease onset, starting at day 11 after immunization, and a severe form of CIA. In contrast, lower disease incidence with later onset (day 18) and lower severity of articular inflammation were observed in control mice after one single immunization with CII. Consistent with clinical findings, inflammation, cartilage erosion and neutrophil infiltration in IL-1RaDM mice were markedly increased as compared to control mice. The ex vivo proliferation of draining lymph node (DLN) cells to CII was significantly increased in cells isolated from IL-1RaDM mice as compared to control mice, suggesting a higher sensitivity to CII in conditional KO mice. Interestingly, IFNγ production was significantly enhanced, and IL-17 production tended to be increased in CII stimulated-DLN cells isolated from IL-1Ra-DM mice versus control mice. Surprisingly, in spite of myeloid cell-derived IL-1Ra deficiency, IL-1Ra levels in arthritic joints of IL-1RaDM mice were significantly higher than in joints of control mice.

Conclusions: The results suggest that myeloid cell-derived IL-1Ra plays a major role in the control of the immune response and in both development and severity of CIA. We propose that excess IL-1 signalling in DLNs, due to IL-1Ra deficiency, leads to enhanced IFNγ and IL-17 productions which may in turn contribute to increased joint inflammation and bone destruction. In addition, the results suggest that resident cells such as chondrocytes and synovial fibroblasts contribute to the local production of IL-1Ra in arthritic joints of IL-1RaDM mice.

P50

Biological role of hepatocyte-derived interleukin-1 receptor antagonist in a model of systemic inflammation

Lamacchia C., Palmer G., Benoit A., Talabot-Ayer D., Gabay C.
University of Geneva School of Medicine (CMU), Division of Rheumatology and Department of Pathology, Geneva, Switzerland

Interleukin-1 receptor antagonist (IL-1Ra) is a specific interleukin-1 (IL-1) inhibitor that possesses anti-inflammatory activities in experimental models and in patients. Hepatocytes produce IL-1Ra in large amounts in response to inflammatory stimuli as an acute-phase protein and were thus suggested to represent a major source of circulating IL-1Ra during systemic inflammation. In addition, IL-1Ra deficient mice (IL-1Ra KO) have an increased susceptibility to LPS-induced death.
Objectives: The aims of this study were to determine the contribution of hepatocytes as cellular source of circulating IL-1Ra in the control of systemic inflammation induced by lipopolysaccharide (LPS) injection and also to define the functional role of hepatocyte-derived IL-1Ra in the control of LPS-induced lethality.
Methods: Conditional hepatocyte-specific IL-1Ra deficient mice (IL-1RaDH) were generated in a pure C57BL/6 genetic background by using the LoxP / Cre-recombinase system. LPS (2 mg/kg or 10 mg/kg) was injected intraperitoneally (i.p.) into IL-1RaDH and wild-type (wt) mice to induce a systemic inflammatory response and IL-1Ra was quantified by ELISA in liver extracts and sera 4 h or 18 h after injection. LPS (10 mg/kg) was injected i.p. into IL-1RaDH mice, wt mice, and IL-1Ra KO mice for the survival test.
Results: After LPS challenge, IL-1Ra mRNA and protein levels were specifically decreased by 80% in the liver of the conditional KO as compared to wt mice. Surprisingly, the plasma levels of IL-1Ra were decreased only by 30% in conditional KO as compared to wt mice, 4 h after injection of 2 mg/kg LPS. No significant difference was observed 18 h after injection. After injection of 10 mg/kg LPS, the levels of circulating IL-1Ra were decreased by 50% and 66% respectively, after 4 h and 18 h, in conditional KO as compared to wt mice. Finally, IL-1Ra KO mice were more susceptible than control mice to the lethal effects of endotoxin. However, there was no difference in survival between control mice and IL-1RaDH mice.
Conclusions: Hepatocytes can be considered as a major source of IL-1Ra in the liver in response to LPS. In addition, the results indicate that the contribution of hepatocytes as a source of circulating IL-1Ra is LPS dose-dependent, but only partial. Finally, hepatocyte-derived IL-1Ra is not required for survival to endotoxinemia. These observations suggest the presence of other sources of circulating IL-1Ra in this model.

P51

The role of JAM-C in regulation of T cell proliferation

Lee B. P.-L., Bradfield P.F., Ody C., Imhof B.A.
Centre Médical Universitaire, Pathology and Immunology, Geneva, Switzerland

JAM-C is an adhesion molecule initially discovered within the vascular compartment and has been implicated in cell polarization, inflammatory events, cell-to-cell adhesion and leukocyte transmigration. We observed through flow cytometric analysis that JAM-C is also expressed at low levels on CD4 and CD8 T cells. Furthermore, this expression was rapidly upregulated following CD3/CD28 induced activation. Further analysis in conjunction with CFSE staining showed peak JAM-C expression within the first two cell divisions followed subsequently by rapid downregulation. Addition of JAM-C blocking antibody during shear flow transmigration studies did not noticeably affect T cell diapedesis. Similarly, coculture with JAM-C blocking antibodies during activation did not appear to affect T cell proliferation. Addition of a soluble form of the high affinity JAM-C ligand, JAM-B, also had no affect on proliferation. However, when CD4 or CD8 T cells were activated in the presence of soluble JAM-C, significant inhibition of proliferation was observed. These results suggest that JAM-C expression on activated T cells may be involved in regulation of immune response. Furthermore, we identify here a new function for JAM-C in inhibition of T cell proliferation. The mechanism by which this activity is achieved requires further study.

P52

Schistosoma mansoni TOR: a potential vaccine target

Lochmatter C., Schifferli J.A., Martin P.J.
University Hospital Basel, Department of Biomedicine, Basel, Switzerland

A trispanning orphan receptor (TOR) has been described in Schistosoma haematobium and Schistosoma mansoni. Here we report the complete molecular organisation of the Schistosoma mansoni TOR gene, also known as SmCRIT (complement C2 receptor inhibitor trispanning). SmTOR gene consists of four exons and three introns as shown by cloning the single exons from S. mansoni genomic DNA and the corresponding cDNA from the larval stage (cercaria) and the adult worm. The SmTOR ORF consists of 1260 bp and is longer than previously reported with a fourth transmembrane domain (proposed new name: Tetraspanning Orphan Receptor), with however an unchanged C2 binding domain on the extracellular domain one (ed1). This domain differs in Schistosoma japonicum. A protein at the approximate expected molecular weight (55 kDa) was detected in adult worm extracts with polyclonal and monoclonal antibodies. Developmental expression profiling showed SmTOR expression to be highest in cercariae, where we were able to detect it on the surface. The higher expression in cercariae as compared to adult worm is of interest since cercariae are in first contact with human skin. SmTOR might have a function in determining the fate of the infection at this time point. In addition, SmTOR being an antigen at the surface of cercariae may be a good target for vaccine development.

P53

Histone deacetylase inhibitors (HDIs) impair cytokine production by interfering with NF-kB signalling in macrophages

Lugrin J., François P., Schrenzel J., Calandra T., Roger T.
CHUV, Infectious Diseases, BH 19-111, Lausanne, Switzerland

HDIs are powerful anticancer drugs with anti-inflammatory properties which have recently been introduced in the clinic. We have previously reported that trichostatin A (TSA) and valproic acid (VPA), two prototypical HDIs, inhibit the response of innate immune cells to microbial stimulation and protect mice from lethal Pam3CSK4 lipopeptide-induced toxic shock. Here we analyzed the molecular mechanisms by which HDIs interfere with macrophage response to microbial stimulation.
Methods: Macrophages were pre-incubated with TSA and VPA and then stimulated with ultra pure LPS and Pam3CSK4. The expression of cytokines and the activation of intracellular signalling pathways were analyzed using DNA arrays, RT-PCR, ELISA, EMSA and Western blotting. NF-kB-dependent transcriptional activity was measured in RAW 264.7 macrophages transiently transfected with a multimeric kB-luciferase reporter vector.
Results: TSA and VPA strongly inhibited the production (mRNA and protein levels) of cytokines (TNF, IL-6, IL-12p40, IL-10) and chemokines (CXCL10, CCL2) induced by LPS and Pam3CSK4 in macrophages. Unexpectedly, HDIs did not interfere with stimulus-induced nuclear translocation of NF-kB p65 and phosphorylation of ERK1/2 and p38 MAPKs, IRF3 and STAT-1, suggesting that HDIs do not inhibit intracellular signal transduction pathways. Moreover, TSA strongly increased the acetylation of TNF, IL-6 and IL-12p40 promoter associated H4 histones, a modification usually linked with active gene transcription. Even so, TSA impaired the recruitment of RNA polymerase II and NF-kB p65 to the TNF, IL-6 and IL-12p40 bona fide promoters and inhibited NF-kB-dependent luciferase activity in macrophages.
Conclusions: HDIs inhibit macrophage response to microbial stimulation, at least in part, by targeting NF-kB p65 DNA binding activity, which may result from an increased acetylation of p65, the recruitment of transcriptional repressors or modifications of chromatin structure.

P54

Distinct patterns of T cell dynamics during extravasation across different types of brain derived endothelium

Lyck R., Steiner O., Makrides V., Verrey F., Engelhardt B.
Universität Bern Theodor Kocher Institut, Bern, Switzerland

T cell extravasation across the blood brain barrier (BBB) endothelium is essential for immunosurveillance of the central nervous system (CNS). However, prevention of T cell accumulation within the brain or spinal chord is a strong ambition for the development of pharmaceuticals to combat inflammatory diseases of the CNS.
To provide essential knowledge on the molecular details of T cell extravasation across the highly specialized BBB endothelium we depend on brain derived endothelial cells (ECs) for our studies. Previously, we used brain derived immortalized endothelioma cell lines to study the molecular details of T cell extravasation. Since these endotheliomas do not form tight monolayers resembling those of the BBB endothelium in vivo, we have extended our studies to an in vitro

BBB model consisting of primary mouse brain microvascular endothelial cells (pMBMECs).
Primary MBMEC monolayers form a tight barrier for water-soluble molecules. Immunofluorescence stainings and quantitative polymerase chain reaction (PCR) showed expression and proper localization of tight junctional proteins resembling TJs of the BBB in vivo. An Affymetrix gene chip array analysis for non-cultured BBB endothelium, pMBMECs cultured for 5 days and immortalized ECs was carried out to compare the transcriptome of endothelial molecules involved in inter-endothelial junction formation or T cell interaction with the endothelium. T cell diapedesis across pMBMECs and immortalized ECs was compared with T cell adhesion to both types of EC monolayers. Finally, live cell imaging was applied to study the dynamics of T cell extravasation across these monolayers.

P55

JAM-B regulates leukocyte tumor infiltration

Meguenani M., Garrido-Urbani S., lee B.P.-L., Imhof B.A.
CMU, faculté de médecine de Genève, Department of Pathology and Immunology, Geneva 4, Switzerland

Tumor growth and angiogenesis are driven by inflammatory ractions mediated in part by leukocytes. The recruitment of leukocytes from the circulation to sites to inflammatory tumors requires sequential engagemment of adhesion receptors and leads to transendothelial migration of the leukocytes. The junctional adhesion molecule B (JAM-B) is localized at interendothelial junctions and is a receptor for JAM-C. We performed in vivo tumor graft experiments using Lewis Lung Carcinoma Cells. Mice were treated with an anti-JAM-B monoclonal antibody which blocks JAM-B/JAM-C interactions. This treatment increased the number of intratumoral, resident type monocytes and decreased the number of inflammatory type monocytes. Interestingly, the antibody also decreased CD8 and regulatory T cells (CD4+CD25+) in the tumors. These results correlate with tumor weight as the treated mice develop smaller tumors compared to the non treated animals. In general, our study demonstrates a new mechanism of action of the immune system which is related to the pro-inflammatory function but not dependent on antigen presentation and its influence on tumor growth.

P56

LY49D engagement on T lymphocytes induces TCR-independent activation and CD8 effector functions that control tumor growth

Merck E., Voyle R. B., MacDonald H.R.
Ludwig Institute for Cancer Research, Lausanne Branch, University of Lausanne, Epalinges, Switzerland.

Recent data showing expression of activating NK receptors (NKR) by conventional T lymphocytes raise the question of their role in the triggering of TCR-independent responses, which could be damaging for the host. Transgenic mice expressing the activating receptor Ly49D/DAP12 offer the opportunity to better understand the relevance of ITAM signalling in the biology of T cells. *In vitro* experiments showed that Ly49D engagement on T lymphocytes by a cognate MHC class I ligand expressed by CHO cells or by specific antibody triggered cellular activation of both CD4 and CD8 populations with modulation of activation markers and cytokine production. The forced expression of the ITAM-signalling chain DAP12 is mandatory for Ly49D transgenic T cell activation. In addition, Ly49D stimulation induced T lymphocyte proliferation, which was much stronger for CD8 T cells. Phenotypic analysis of anti- and to kill target cells indicate that Ly49D ligation generates effector cytotoxic CD8 T cells. Ly49D engagement by itself also triggered cytotoxic activity of activated CD8 T cells. Adoptive transfer experiments confirmed that Ly49D transgenic CD8 T cells are able to control growth of CHO tumor cells or RMA cells transfected with Hm1-C4, the Ly49D ligand normally expressed by CHO. In conclusion, Ly49D engagement on T cells leads to T cell activation and to a full range of TCR-independent effector functions of CD8 T cells.

P57

Climbing stairs increases peripheral blood NK cell counts, but reduces CD56^{bright} frequency and NK cell activation by IL2 and TLR2 agonists

Millard A.L., Valli P.V., Stussi G., Mueller N. J., Seebach J. D.
University Hospital Zurich, Department of Internal Medicine, Zürich, Switzerland

Regular physical activity is associated with a reduced risk of mortality. Exercise has both positive and negative effects on immune functions. Following episodes of prolonged, continuous heavy exercise, the circulating numbers and functional capacity of leukocytes is temporarily impaired. Here, we investigated whether climbing stairs can influence the number and function of circulating NK cells. To address this question, peripheral blood was drawn from 24 healthy volunteers immediately before and after sprinting up and down 150 stair steps. The total cell numbers of all leukocyte subsets (neutrophils, lymphocytes, monocytes, eosinophils and basophils) rose upon exercise. The number of PBMC and NK cells obtained by Ficoll isolation of post-exercise blood samples increased by 2.3-fold, and 6.3-fold, respectively. Concomitantly, the frequency of CD56^{bright} NK cells was 2.9-fold lower. Exercise did not directly influence NK cell function, since no difference were observed in IFNγ secretion and cytotoxicity between NK cells isolated pre- or post-exercise. However, post-exercise activation of NK cells with IL2 or TLR2 agonists (lipoteichoic acid and Pam3CSK4) led to an up to 3-fold reduced IFNγ secretion. The frequency of IFNγ secreting NK cells was also decreased post-exercise. Finally, both cytokine and TLR agonist triggered NK cytotoxicity was impaired post-exercise. Taken together these data suggest that, by interfering with NK cell activation, exercise might have major implications on NK cell-mediated immunity.

P58

Role of TREM-1 in mediating monocyte/macrophage differentiation and functions

Weber B., Schenk M., Reith W., Mueller C.
Institute of Pathology, University of Bern, Bern, Switzerland

Triggering receptor expressed on myeloid cells (TREM)-1 is implicated in the amplification of inflammatory responses by enhancing degranulation and secretion of pro-inflammatory mediators. Recent results obtained with specific antagonistic peptides that block TREM-1 binding to its ligand(s) confirm the potential of TREM-1 as a target to prevent excessive inflammation also of chronic inflammatory disorders. Based on the differential expression of several cell surface markers, monocytes are generally divided into an “inflammatory“ and a “resident“ monocyte population. We now observed that in the so-called inflammatory type mouse monocyte subset only a minor fraction (less than 10%) are positive for cell surface expression of TREM-1 whereas in the so-called resident subset TREM-1 positive, and –negative monocytes are present at equal frequencies. Although preliminary in nature, these results clearly demonstrate the heterogeneity of these two monocyte subsets and the need for their further functional and phenotypic characterisation. We hypothesize that the analysis of TREM-1 expression, together with the use of additional markers such as CX3CR1 will allow to further dissect the monocyte/macrophage cell populations into functionally distinct subsets and will possibly also allow to gain information on the lineage relationship of these monocyte/macrophage populations. To more closely determine the relevance of TREM-1 mediated effects in acute and chronic inflammation we started to generate a conditional TREM-1 knockout mouse, which may also provide insight into the plasticity of the monocyte/macrophage lineage, particularly, during inflammatory conditions. A TREM-1 knockout mouse will allow to directly evaluate the potential of TREM-1 targeting therapies and allow to assess the contribution of TREM-1 in a wide variety of disorders with proven, or anticipated, involvement of myeloid cells including inflammatory bowel diseases, experimental allergic encephalomyelitis, chronic transplant rejection and atherosclerosis.

P59

Anti-CD154 mAb and rapamycin mediate early regulation in xenogeneic islet transplantation

Muller Y., Golshayan D., Seebach J., Mai G., Morel Ph., Serre-Beinier V., Gonelle C., Puga Yung G., Wyss J.-C., Bigenzahn S., Wekerle Th., Bühler L.H.
Internal Medicine, Clinical immunology, Genève 14, Switzerland

Combined therapy with anti-CD154 monoclonal antibody (mAb) or MR1 and rapamycin (RAPA) was shown to induce indefinite survival of concordant rat-to-mouse islet xenografts. The aim of the present study was to investigate whether regulation by CD25+Foxp3+ regulatory T cells (Treg) played a role in the induction and maintenance of the observed tolerance.
Principal findings: Anti-IL2 mAb or depleting anti-CD25 mAb induced rejection (100% and 89% respectively) when administered early together with MR1 and RAPA. In contrast, when anti-IL2 mAb or anti-CD25 mAb were given late, only a minority of xenograft recipients rejected (25% and 40% respectively). Analysis of blood, spleen, para-aortic lymph nodes and graft showed an early increase of Treg in graft and lymph nodes of tolerant mice. In contrast rejecting mice showed a transient increase of Treg which disappeared within 48 hours – 7 days after graft destruction. No significant increase of Treg was observed 100 days post transplantation in tolerant mice.
Conclusion: In conclusion, tolerance induction by RAPA and MR1 for concordant islet xenografts was characterized by early presence of Treg. Tolerance was reverted by anti-IL2 mAb or anti-CD25 mAb treatment at the time of transplantation. Treg may play a critical role for xenograft acceptance early after rat-to-mouse islet transplantation.

P60

Dissecting the role of T helper cells in autoimmune myocarditis

Nindl V., Maier R., De Giuli R., Miller S., Züst R., Waisman A., Rülicke Th., Thiel V., Ludewig B.
Research Department, Cantonal Hospital St. Gallen, St. Gallen, Switzerland

Cardiovascular diseases like myocarditis and subsequent dilated cardiomyopathy (DCM), are a frequent cause of mortality in humans with DCM being the most common reason for heart failure in young adults. Infections with *Coxsackievirus B3* or *Cytomegalovirus* can lead to an acute inflammation of the heart muscle that is followed by an autoimmune response directed autoantigens in the heart, such as the isoform of cardiac myosin (myhca). Immunization with the well-characterized myhca$_{614-629}$ epitope elicits autoreactive CD4$^+$ T cell responses that have been shown to be the major mediators of autoimmune myocarditis in Balb/c mice. It is known that professional antigen presenting cells (APCs) such as dendritic cells are crucial for initiating and maintaining Th responses affecting the heart muscle. However, the detailed analysis of the interaction between these cells in the context of autoimmune myocarditis has been hampered by the lack of appropriate analytical tools. We therefore generated a TCR transgenic mouse harbouring T cells that specifically recognize the myhca$_{614-629}$ peptide. In a first step, hybridoma cells were generated by fusing BW5147 TCRa$^-$CD8$^-$ lymphoma cells with myhca$_{614-629}$-specific Th cells. TCR expression and antigen specificity was assessed by FACS analysis and ELISPOT assay. Following subcloning, the variable regions of the expressed TCR were characterized by PCR-sequencing. The rearranged V(D)J regions were subcloned into TCR cassette vectors and linearized constructs were injected into the pronuclei of fertilized oocytes. Using this novel TCR tg mouse we plan to investigate in detail the activation of myhca$_{614-629}$-specific T cells during the process of autoimmune myocarditis. Furthermore, this new tool will help to generate a high resolution analysis of the contribution of different APCs in the activation and differentiation of autoreactive Th cells during inflammatory heart disease.

P61

A novel anti-inflammatory role of TNFα in intestinal inflammation by inducing local glucocorticoid synthesis

Noti M., Corazza N., Berger B., Brunner Th.
Pathology, Immunopathology, Bern, Switzerland

TNFα is a cytokine with most prominent pro-inflammatory action. Its critical role in the pathogenesis of various inflammatory diseases has led to the development of different TNF-based therapies. In particular, inflammatory bowel disease, such as Morbus Crohn, is successfully treated with anti-TNFα. However, there is increasing evidence that TNFα has also important anti-inflammatory activities. Glucocorticoids are important immunoregulatory steroids. We recently described that glucocorticoids are produced in the intestinal epithelium in an adrenal gland-indepedent manner and contribute to the maintenance of local immune homeostasis. Here we show that induction of experimental inflammatory bowel disease strongly induces the expression of steroidogenic enzymes and the production of glucocorticoids in the intestinal mucosa. The induction of intestinal steroidogenesis was strongly dependent on the type of inflammatory response. While Th1-type of inflammation promoted intestinal glucocorticoid synthesis, a Th2-type of inflammation failed to do so. This inflammation-induced induction of steroidogenesis was attributed to the presence of TNFα. Direct administration of TNFα strongly induced intestinal steroidogenesis, likely by directly inducing the expression of steroidogenic enzymes in intestinal epithelial cells. Critically, therapeutic administration of TNFα was able to restore intestinal glucocorticoid synthesis in a Th2-type of inflammation, and thereby to ameliorate the pathogenesis of inflammatory bowel disease. These data describe a novel anti-inflammatory role of TNFα in the pathogenesis of inflammatory bowel disease via the induction of local steroidogenesis.

P62

Stromal cell specific transgenic mouse models for a deepened understanding of the T cell zone stroma

Onder L., Scandella E., Züst R., Mayer Ch., Sparwasser T., Ludewig B.
Laborforschungsabteilung LFA, Kantonsspital St. Gallen, St. Gallen, Switzerland

The stromal cell network plays a key role in the development of lymphoid tissues and in lifelong maintenance of secondary lymphoid organ structure. Stromal cell- specific expression of the constitutive chemokines CCL13, CCL19 and CCL21 ensures controlled lymphocyte attraction and therefore organizes the microenvironment of lymphoid organs. Many viral infections, such as human immunodeficiency virus or measles virus infections are associated with an immunopathological destruction of the lymphoid organ microenvironment. A recent study from our laboratory has shown that infection of mice with the lymphocytic choriomengitis virus leads to destruction of secondary lymphoid organ structure that is mediated by virus-specific cytotoxic T cells. Furthermore, stromal cell – lymphoid tissue inducer (LTi) cell interaction was shown to be crucial for restoration of adult secondary lymphoid organ integrity after acute LCMV infection (Scandella et al, Nature Immunology, 2008). However, the cellular and molecular mechanisms underlying LTi cell – stromal cell interaction and stromal cell function in general remain to be characterized in more detail. The aim of this project is to analyze stromal cell functions *in vivo* using novel transgenic mouse models. To this end, the gene of the Cyclization Recombinase (Cre) will be expressed under the control of constitutive promoters (ELC/CCL19 and podoplanin/gp38) that are active in stromal cells. Future experiments will involve Cre inducible reporter gene expression, targeted stromal cell depletion and stromal cell-specific antigen presentation in order to further characterize the role of stromal cells during viral infections.

P63

Pro-interleukin(IL)-33 is biologically active independently of caspase-1 cleavage

Palmer G., Talabot-Ayer D., Lamacchia C., Gabay C.
University of Geneva School of Medicine, Pathology and Immunology, Geneva 4, Switzerland

The new IL-1 family cytokine IL-33 is synthesized as a 30kD precursor. Human pro-IL-33, like pro-ILβ, is cleaved by caspase-1 in vitro to generate a 17kD fragment, which is sufficient to activate signaling by the IL-33 receptor T1/ST2. However, the proposed caspase-1 cleavage site is poorly conserved between species. In addition, it is not clear whether caspase-1 cleavage of pro-IL-33 occurs in vivo, and whether, like for IL-1β, this cleavage is a prerequisite for IL-33 secretion and bioactivity. In this study, we further investigated caspase-1 cleavage of pro-IL-33 in vitro and in cultured cells and assessed potential bioactivity of the IL-33 precursor. Lysates of 293T HEK cells overexpressing mouse pro-IL-33 were used for in vitro caspase-1 assays. Pro-IL-33 cleavage upon endogenous caspase-1 activation was examined in THP-1 cells overexpressing human IL-33. Mouse pro-IL-33 purified from 293T lysates or translated using a cell free system was used to stimulate P815 mastocytoma cells or primary mast cells (MC). Mouse pro-IL-33 incubated with active caspase-1 yielded a cleavage product of 20kD, which has a higher molecular weight than the recombinant IL-33 protein commonly used to activate T1/ST2. Caspase-1 activation in THP-1 cells induced cleavage of pro-IL-1β, but not of pro-IL-33, which localized essentially to the cell nucleus. Activated THP-1 cells secreted both cleaved IL-1β and full length pro-IL-33. Addition of pro-IL-33 to P815 cells induced IL-6 secretion, which was inhibited in presence of soluble ST2-Fc. Consistently, incubation with pro-IL-33 induced IL-6 production in wild-type, but not T1/ST2 knockout MC, suggesting that pro-IL-33 exerts T1/ST2 dependent biological effects. In conclusion, cleavage of mouse pro-IL-33 in presence of caspase-1 yields a product, which is different from recombinant 'mature' IL-33. In addition, our results suggest that caspase-1 cleavage is not required for IL-33 secretion and bioactivity.

P64

T regulatory cell generation in human tonsils: a role for plasmacytoid dendritic cells

Palomares O., Rückert B., Romagna A., Speiser S., Akdis M., Akdis A.
Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Immunology group, Davos, Switzerland

Tonsils are strategically located to protect the gateway of both respiratory and alimentary tract and they must discriminate between potentially infectious pathogens and innocuous airborne or food antigens. The aim of this work was to study a component of oral tolerance, the mechanisms of T regulatory cell generation in human tonsils by analyzing the phenotypic and functional properties of tonsil T cell and dendritic cell (DC) subsets. In tonsil mononuclear cells (TMC), FOXP3+ T regulatory cells constitute approximately 10% of the T cell population. Tonsil FOXP3+ T regulatory cells express CD25 (5.1%), CD45RA (1.3%), CD45RO (8.4%), CD62L and CD39 (3.5%) and produce IL-10 and IFNγ as determined by flow cytometry and intracellular staining. The immunosuppressive capacity of purified FOXP3+ T regulatory cells from tonsils was demonstrated in coculture experiments with autologous TMC. Plasmacytoid dendritic cells (pDCs) represented around 65% of the total tonsil dendritic cell population. Freshly purified pDCs showed a partially immature phenotype as they expressed HLA-DR and CD40 but not other activation surface markers such as OX40L, CD80, CD83, PDL1 or ICOSL. After treatment with IL-3, TLR7 and -9 agonists over 24 h, maturing pDCs induced proliferation of allogenic naïve CD4+ T cells. Treg cells colocalize with pDCs in tonsils in close contact to epithelial areas. Real-time quantitative PCR analysis, cytokine levels in supernatants and intracellular staining in maturing pDC/T cell coculture experiments indicated that tonsil pDCs have the ability to induce T regulatory cells from naïve CD4+ T cells. These results show

that pDCs and the generation of Treg cells with tolerogenic properties are taking place in human tonsils, thus establishing cellular basis to suggest that tonsils are secondary lymphoid organs where tolerance induction to food and aero-antigens as well as to sublingual allergen-specific immunotherapy vaccines might well be generated.

P65

Tumour-protective memory CD8+ T cells develop in vivo following in vitro activation by mature DC

Perret R., Yang J., Franca Ronchese F.
Malaghan Institute of Medical Research, Current contact details: LICR Lausanne Branch, Lausanne, Switzerland

Inducing functional and durable T cell memory is critical to the success of immunotherapy. This is particularly important in the therapy of cancer where immune responses to tumours can be weak and short-lived, providing inefficient protection. We used the line 318 TCR-transgenic mouse strain, in which the CD8+ T cells recognise the LCMV gp33-41 peptide in the context of H-2Db, to study memory development of in vitro activated T cells. Line 318 T cells were activated by culture with mature DC and specific antigen and maintained in IL-2. They proliferated extensively, became CD62L-, secreted IFNγ, TNFα and IL-2 upon restimulation and acquired cytotoxic activity. In vitro activated effector T cells transferred intravenously into CD45 congenic animals were detectable in all tissues examined, both immediately after i.v. transfer and following a rest period in the absence of antigen. The donor T cells acquired a memory phenotype over time. They were heterogeneous in CD62L expression and expressed IL-7Rα and IL-15Rβ, as well as undergoing homeostatic proliferation. They maintained their ability to produce multiple cytokines in response to specific antigen and remained KLRG-1- following both primary and secondary activation. Prophylactic T cell transfer protected mice from challenge with either B16.GP33 or LL-LCMV tumour cells for up to 90 days. In addition, the transfer of large numbers of T cells delayed the growth of established B16.GP33 tumours in a therapeutic setting. These results indicate that, given the right in vitro activation conditions, protective memory CD8+ T cells can be generated for use in immunotherapy.

P66

Allogenic MSC impairs CD107 upregulation, perforin release and cytotoxicity of in vitro stimulated natural killer cells

Pradier A., Villard J., Passweg J., Kindler V.
Geneva University Hospital, Hematology, Geneva 14, Switzerland

Mesenchymal pluripotential stem cells (MSC) differentiate into various lineages including osteoblasts, chondrocytes and adipocytes, and prolong skin allograft via a MHC-independent mechanism. As alloreactive T lymphocytes (TL) and natural killer cells (NK) participate in graft rejection, one may expect that MSC may target both cell types. MSC-induced TL inhibition is partly mediated by indolamine 2,3 oxygenase (IDO), a L-tryptophan (TRP) degrading enzyme that is activated in MSC by interferon γ. The effect of MSC on NK function and the eventual contribution of IDO in such a process are so far poorly understood. We therefore investigated these issues. Culture of NK with interleukin (IL)-15 for 5 days induced an extensive proliferation (83% of CFSE low cells generated vs. 0.3% in absence of cytokine). IL-15 pre-activated NK subsequently killed MSC and K562 cells with an efficiency of 12 and 44% respectively (E:T ratio of 1:1). By contrast when NK were co-cultured with MSC in presence of IL-15 the fraction of CFSElowCD56+ cells dropped to 35% ($p < 1xE-6$; $n = 13$). NK lysed a significant fraction of MSC during the co-cultures with IL-15. However, NK derived from such cultures exhibited a decreased ability to upregulate CD107 expression on cell surface and to release intracellular perforin, and a decreased cytotoxic activity when incubated with K562 (15%, $p < 1xE-8$, $n = 16$). These alterations were not observed upon activation of NK in the presence of allogenic HUVEC. Supernatants of co-cultures, but not of cultures of NK without MSC contained significant amounts of kynurenine (19 μM $p < 0.016$ $n = 4$) resulting from TRP degradation by IDO. Complementation with exogenous TRP did not overcome MSC-induced NK inhibition, suggesting that kynurenine accumulation rather than TRP depletion may inhibit NK functions. Altogether these data show that allogenic MSC, though sensitive to NK lysis, inhibit NK proliferation and cytolytic activity on a third party target. MSC-induced inhibition may be initiated by kynurenine or other TRP degradation products released after the activation of IDO. These metabolites could in turn mediate the inhibition of NK proliferation and the alteration of the upregulation of CD107 expression and of the release of intracellular perforin leading to a reduced cytotoxicity.

P67

Absence of isoglobotrihexosylceramide 3 and the Gal xeno-antigen in endothelial cells derived from -1,3Galactosyltransferase knock out (GalTKO) pigs

Puga Yung G., Yunsen L., Borsig L., Karpova M., Zhou D., Seebach J.
University Hospital and University of Geneva, Internal Medicine, Immunology and Allergology, Geneva, Switzerland

Anti-Gal-α1,3Gal-β1,4GlcNAc-R (Gal) natural Ab are responsible for hyperacute rejection (HAR) occurring after pig-to-primate xenotransplantation. Although the generation of pigs lacking the Gal transferase (GalT) has overcome HAR, the literature is contradictory regarding the complete elimination of the Gal epitope in GalTKO pigs. One possible candidate that could account for remaining Gal expression is the isoglobotrihexosylceramide 3 (iGb3).
Hypothesis: The terminal Gal-α1,3Gal disaccharide is completely absent in endothelial cells derived from GalTKO pigs.
Material and methods: FACS analysis of Gal and iGb3 in pig aortic endothelial cells (PAEC) derived from wild type (WT) and GalTKO animals using lectins and a panel of anti-iGb3 and -Gal specific Ab. Ion-trap mass spectroscopy (MS) analysis of the glycolipid fractions of WT and GalTKO PAEC membranes. Finally, the GalT enzymatic activity was determined.
Results and conclusion: No GalT enzymatic activity and no surface expression of Gal or iGb3 by Ab staining was found on GalTKO PAEC. Lectin staining showed an increase in the H-type sugar structures present in GalTKO as compared to WT PAEC. Ion trap-MS analysis did not reveal Gal in cellular membranes of GalTKO PAEC, iGb3 was also totally absent, whereas a fucosylated form of iGb3 was detected at low levels in both PAEC extracts. The precise position of the Fucose has not been resolved yet. In summary, the results confirm our hypothesis by two different approaches, using Gal specific Ab, and the sensitive ion-trap MS technique. Thus, we found no evidence for the presence of iGb3, and even if traces of iGb3 were expressed on GalTKO PAEC, they are not relevant for HAR as no Ab-binding was detected. Future work will have to focus on other mechanisms leading to xenograft rejection including non-Gal antibody and cellular responses.

P68

TLR3 ligands as multifunctional adjuvants for melanoma therapy

Romero P., Salaun B., Greutert M., Rimoldi D.
Ludwig Institute for cancer research, Division of clinical onco-immunology, Hôpital orthopédique HO-05, Lausanne, Switzerland

Toll-like receptors (TLR) recognize pathogens derived patterns and are first line players in mounting anti-microbial pro-inflammatory immune responses. These properties may be harnessed for therapeutic purposes, and some TLR agonists are now widely recognized as potent adjuvants for vaccines, including those against tumors. However, their mechanism(s) of action(s) remain(s) largely unknown. In the present work, we demonstrate that double stranded RNA may increase anti-cancer vaccine efficacy in vivo by at least two mechanisms. Using a model of antigen-specific CD8+ T cells adoptive transfer, we showed that the TLR3 agonist Poly(I:C) strongly enhances immune responses to peptide vaccine. This effect was dependent on TLR3, as Poly(I:C) failed to increase the expansion of specific CD8+ T cells when adoptively transfered into TLR3 deficient hosts. Moreover, we established that human melanoma cells can express functional TLR3 protein. Engagement of this receptor by synthetic TLR3 agonists can block cell proliferation and induce apoptosis in tumor cells in vitro, especially after IFNα pretreatment. Most importantly, these results could be translated in vivo, as growth of human melanoma xenografts in fully immunodeficient mice (Rag2 / common γ chain double knockout) could be strongly inhibited by a systemic treatment consisting of combined injections of human IFNα and Poly(I:C). This immune system independent anti-tumor effect of TLR3 agonists was partly mediated by TLR3 expressed by the tumor cells themselves, since melanoma cells stably depleted of TLR3 protein by lentivirus-mediated siRNA expression were less sensitive to Poly(I:C)-induced growth inhibition in immunodeficient hosts. Altogether, these results indicate that TLR3 agonists represent promising multifunctional adjuvants for cancer vaccines, not only based on their immunostimulatory properties, but also due to their direct pro-apoptotic effect on tumor cells.

P69

Role of junctional adhesion molecule a (JAM-A) in the immunopathogenesis of experimental autoimmune encephalomyelitis (EAE)

Schäfer J., Boscacci R., Adams R., Dejana E., Imhof B. A., Engelhardt B.
University of Bern, Theodor-Kocher Institut, Bern, Switzerland

In multiple sclerosis (MS), and in its animal model, experimental autoimmune encephalomyelitis (EAE), circulating immune cells gain access to the central nervous system (CNS) and cause inflammation, blood-brain barrier (BBB) breakdown and demyelination, which all set

the stage for the development of this disabling disease. Inflammatory cell recruitment across the BBB has been recognized as a major pathophysiological hallmark of MS and blocking leukocyte/endothelial interactions has proven to be beneficial for the treatment of this disease. But the molecular mechanisms involved in diapedesis, the last step of the multi-step leukocyte recruitment across the BBB, are not yet understood very well. The junctional adhesion molecules A (JAM-A), JAM-B and JAM-C have been suggested to mediate leukocyte diapedesis asross the vascular wall. Here we investigate the potential involvement of JAM-A in the immunopathogenesis of EAE with special focus on leukocyte trafficking across the BBB using JAM-A deficient mice. Besides its documented expression on immune cells immunofluorescence stainings of CNS cryosections using a monoclonal antibody confirmed expression of JAM-A by endothelial cells of the BBB. EAE was induced by immunization of WT and JAM-A deficient C57BL/6 mice with the MOG35-55 peptide in CFA. JAM-A deficient mice showed an ameliorated disease course compared to wildtype mice, supporting an involvement of JAM-A in EAE pathogenesis. JAM-A was found to have neither an influence on the priming of MOG-specific encephalitogenic T lymphocytes nor on BBB permeability. A possible involvement of JAM-A in inflammatory cell diapedesis across the BBB is currently investigated.

P70

Purinergic control of T cell activation by ATP released through pannexin-1 hemichannels

Schenk U., Westendorf A.M., Radaelli E., Casati A., Fumagalli M., Verderio C., Buer J., Scanziani E., Grassi F.
Institute for Research in Biomedicine, T cell development, Bellinzona, Switzerland

T cell receptor (TCR) stimulation results in the influx of Ca2+, which is buffered by mitochondria and promotes ATP synthesis. We found that ATP released from activated T cells through pannexin-1 hemichannels activated the purinergic P2X receptor (P2XR) to sustain mitogen-activated protein kinase (MAPK) signaling. P2XR antagonists, such as oxidised ATP (oATP), blunted MAPK activation in stimulated T cells, but did not affect the nuclear translocation of nuclear factor of activated T cells, thus promoting T cell anergy. In vivo administration of oATP blocked the onset of diabetes mediated by anti-islet TCR transgenic T cells and impaired the development of colitogenic T cells in inflammatory bowel disease. Thus, pharmacological inhibition of ATP release and signaling could be beneficial in treating T cell-mediated inflammatory diseases.

P71

Toll like receptor ligands as adjuvants for VLP-induced T cell responses: different outcomes for different ligands

Schwarz K. , Jäger P., Bachmann M.F.
Cytos Biotechnology AG, Immunodrugs, Schlieren, Switzerland

VLPs are highly repetitive, non replicating particles that induce very strong B cell responses in the absence of adjuvant. The coat protein of the RNA phage Qβ, expressed recombinantly in E.coli, self assembles into VLPs. During this process, E.coli- derived RNA binds to the natural RNA-bindig sites of the coat protein and is included into the particles. Chemical coupling of antigens to the surface of the VLPs renders these antigens highly immunogenic for B cells. In this study, we used the LCMV derived CD8+ T cell epitope p33, coupled to Qβ, to evaluate the CTL-inducing potential of VLPs in mice. The RNA-containing VLPs were found to induce CD8+ T cell expansion, even in the absence of adjuvant. The immune response, however, was insufficient to cope with an infection with recombinant vaccinia virus expressing p33, despite viral titers being slightly reduced. No significant CD8+ T cells response was induced if the E.coli derived RNA was removed from the VLP. Conversely, adding CpG oligonucleotides to the VLP strongly enhanced its immunogenicity, resulting in a vigorous expansion of specific CD8+ T cells that fully protected from the viral infection. RNA and CpGs are ligands of toll like receptors (TLRs) 7/8 and 9, respectively. TLRs are known as potent stimulators of innate immunity. Screening of further TLR ligands revealed a surprising phenomenon: while ligands for TLR3, 7 and 9 enhanced VLP-induced CD8+ T cell responses, TLR2 ligands inhibited rather than enhanced the VLP driven immune response in an IL-10 dependend manner. Thus, dependent on the TLR engaged, VLP-induced immune responses may be strongly enhanced, slightly enhanced or even inhibited.

P72

Building a lymph node in vitro

Siegert S., Britschgi M.R., Tomei A.A., Acha-Orbea H., Swartz M.A., Luther S.A.
University Lausanne, Biochemistry, Epalinges, Switzerland

Adaptive immune responses are only efficiently initiated in the T-zone of secondary lymphoid organs. The encounters of antigen-specific T-cells with antigen-bearing dendritic cells (DC) in this zone are facilitated by a 3-dimensional (3D) network of T zone reticular cells (TRCs) which physically guide T and B cells throughout this zone. In addition, TRCs promote DC and T cell attraction into the T zone by producing the two CCR7 ligands, CCL19 and CCL21. Importantly, TRCs produce the cytokine IL-7, a critical survival factor for naïve T cells. Therefore it has become apparent that TRCs play an important role in adaptive immunity. However, their biology and precise role in T cell priming are poorly understood. An in vitro system reconstructing lymph node (LN) T zones could be of great help to further dissect the process of T cell priming. To this end, we established immortalized murine TRC lines from peripheral LN. These cells express a surface profile comparable to ex vivo TRCs and provide an easy source of cells. When grown in a collagen-containing sponge they build their characteristic 3D-network and show a morphology comparable to their counterparts in LN. By co-culturing T cells and DCs together with the TRCs in this 3D system we will be able to study T cell priming in a close to physiological context. This reconstructed T zone is easily accessible to analysis by different approaches, such as microscopy or flow cytometry. Furthermore, the ease of experimental manipulation of each component will make this system ideal to obtain new insights in the role of TRCs in T cell priming.

P73

Endothelial ICAM-1 and ICAM-2 are involved in T cell crawling on and diapedesis across blood brain barrier endothelium

Steiner O., Coisne C., Cecchelli R., Deutsch U., Engelhardt B., Lyck R.
Theodor-Kocher Institute, University of Bern, Bern, Switzerland

T cell extravasation across the blood brain barrier (BBB) endothelium is essential for immunosurveillance of the central nervous system (CNS). However, prevention of T cell accumlation within the brain or spinal chord is a strong ambition for the development of pharmaceuticals to combat inflammatory diseases of the CNS.
To provide essential knowledge on the molecular details of T cell extravasation across the highly specialized BBB endothelium, we depend on brain derived endothelial cells for our studies. Previously, using brain derived immortalized endothelioma cell lines we demonstrated that endothelial intercellular adhesion molecule-1 (ICAM-1) and ICAM-2 are essential for T cell diapedesis in vitro. Since brain endotheliomas do not form tight junctions resembling those of the BBB in vivo, we have extended our studies to an in vitro BBB model consisting of primary mouse brain microvascular endothelial cells (pMBMECs). Comparing T cell diapedesis across wild-type pMBMECs and ICAM-1/-2 double deficient pMBMECs confirmed the essential role of endothelial ICAM-1 and ICAM-2 in this process. Using this model the individual contributions of endothelial ICAM-1 and ICAM-2 in T cell crawling, a preceeding step before diapedesis across the BBB, are currently investigated. Time lapse videomicroscopy under flow conditions demonstrates differences in the migratiory behavior of T cells on wild-type versus ICAM-1 or ICAM-2 or ICAM-1/-2 deficient pMBMECs. Our study provides evidence for the differential contribution of endothelial ICAM-1 and ICAM-2 in T cell crawling and subsequent diapedesis across the BBB.

P74

TGFβ inhibits expression of IL-27 in macrophages

Trogrlic I., Malipiero U., Ackermann F., Suter T., Reith W., Fontana A.
University Hospital Zurich, Immunology clinic, Zurich, Switzerland

TGFβ is a potent regulatory cytokine with diverse effects on hemapoietic cells and it has an important role in control of autoimmunity. In T cell-mediated autoimmunity, studied on the experimental autoimmune encephalomyelitis model (EAE), it has been shown that TGFβ is increased during the remission phase. Treatment of mice with TGFβ1 ameliorates EAE, whereas anti-TGFβ antibodies lead to exacerbation of EAE. One of the effector cells in EAE are macrophages (Mph) which are thought to participate in myelin damage. Whether TGFβ is also essential in the control of EAE, not only by acting on T cells but also by regulating the function of Mph, remains to be established. In-vitro studies show TGFβ to be a powerful Mph deactivator by inhibition of proinflammatory cytokines such as TNFα and IL-1β. Furthermore, TGFβ impairs the production of oxygen radical intermediates and the generation of nitric oxide (NO) by the inducible NO synthase. In order to dissect the role of TGFβ in the innate immune system and in autoimmunity, we use knockout mice with the specific deletion of the TGFβRII gene in polymorphonuclear leukocytes (PMN) and Mph. Previously, we found that in the absence of TGFβRII signalling in myeloid cells, the chronic phase of EAE takes a more severe course. Unexpectedly, the phenotype was not associated with more prominent expression of iNOS, TNFα and IL-1 in Mph derived from TGFβRII knockout mice. Interleukin 27 (IL-27) produced by antigen-presenting cells is also a key player in autoimmune diseases but its regulation is still not well described. Our current in vitro study shows that IL-27 expression, induced by various stimuli, can be inhibited by TGFβ in mouse Mph isolated from control mice, but not in Mph lacking TGFβRII.

P75

Hematopoiesis "in a dish" on delta-like-1 transduced OP9 monolayers to analyse signals/molecules involved in the development of human T lymphocytes and dendritic cells

Vanhecke D., Dzionek A., Rolink A.
Center of Biomedicine, Developmental and Molecular Immunology, Basel, Switzerland

The importance of normal T lymphocyte development in the immune system is exemplified by the occurrence of inherited and acquired human immunodeficiencies where the development or functional maturation of T cells is defective. In order to identify molecules/genes and elucidate developmental processes that are essential for human T cell development we use a novel in vitro tool, the OP9-DL1 cell culture system. Using this in vitro assay we obtain human cyCD3+ and CD4+CD8+ double positive thymocytes starting from Umbilical Cord Blood (UCB) derived CD34+ hematopoietic stem cells. Signals and molecules that are involved in T cell development are being addressed by using blocking antibodies and/or chemical inhibitors. Similar as in mice we found an essential role for IL-7 and Notch mediated signaling in the development and survival of particular developmental stages of human thymocytes. In addition, by knocking down genes in the CD34+ precursors using RNA interference we will test particular genes for their role during human lymphoid development. In parallel to T lymphocytes, we also obtain dendritic cells (DC), myeloid cells and CD56+ Natural Killer (NK) cells from UCB CD34+ cells in the OP9 and/or OP9-DL1 co-cultures. Since these cells have also been documented in the thymus, the specialized organ where T lymphocytes normally develop in vivo, we have initiated the phenotypic and functional characterization of these non-T-cells. From a clinical standpoint, the identification of those CD34+ HSCs that efficiently give rise to T cells in vitro and in vivo could lead to better allogeneic bone marrow transplantation protocols and consequently in a significant reduction in treatment related mortality rates. Finally, a better understanding of the mechanisms controlling human T-cell development is a fundamental step towards the development of specific therapies for the treatment of primary and acquired immunodeficiencies as well as for the treatment of malignant T-cell disorders.

P76

Novel function for interleukin-7 in dendritic cell development

Vogt T.K., Link A., Perrin J., Finke D., Luther S.A.
Department of Biochemistry, University of Lausanne, Epalinges, Switzerland

Interleukin-7 (IL-7) is crucial for the development of T and B lymphocytes from common lymphoid progenitors and for the maintenance of mature T lymphocytes. The role of IL-7 for dendritic cells (DC) has been poorly defined. We investigated the effect of IL-7 for the development and maintenance of different DC types in vitro and in vivo. DCs generated in vitro from bone marrow cells (BMDC) expressed the IL-7 receptor (IL-7R) and survived significantly longer in the presence of IL-7. Using various mouse models, we found IL-7R to be intrinsically required for cDC and pDC development. As common lymphoid but not myeloid progenitors in bone marrow depend on IL-7, we propose that a considerable fraction of cDCs and pDCs derive from lymphoid-committed progenitors. Together these studies demonstrate that IL-7 not only plays an important role in the development of lymphocytes but also of DCs.

P77

Mechanistic insights into T help-dependent and T help independent CD8 T cell priming

Wiesel M., Agnellini P., Wolint P., Oxenius A.
ETH Zurich, Institute of Microbiology, Zurich, Switzerland

We used an experimental system consisting of CD8+ T cell priming with replication-incompetent virus like particles (VLPs) followed by secondary challenge either with Vaccinia virus (VV), lymphocytic choriomeningitis virus (LCMV) or VLPs, and we found that CD4 T cell help is only crucial for secondary infections with in case of VV challenge. Furthermore, also the primary CD8+ T cell response specific for VV was highly diminished in the absence of T help, indicating that the requirement for T help is rather linked to the type of infection rather than a general feature of secondary CD8+ T cell responses. We investigated whether a co-infection with VV and various other pathogens would overcome the T help requirement for induction of CD8+ T cell responses. To this end, antigen-specific CD8+ T cell were primed by VV in presence ("helped") or absence ("helpless") of T helper cells in combination with a heterologous LCMV, MCMV or *Listeria monocytogenes* infection. Interestingly, co-infection with LCMV completely restored the proliferation of helpless VV-specific CD8+ T cells to the level of helped VV-specific CD8+ T cells, whereas neither MCMV nor *Listeria monocytogenes* infection improved the proliferation capacity of helpless CD8+ T cells. The rescue of helpless CD8+ T cells in this VV heterologous LCMV co-infection was entirely dependent on type I IFN (IFN-I) signaling on CD8+ T cells. It is conceivable that a differential cytokine environment elicited after VV infection versus LCMV infection is related to the T help dependence of CD8+ T cell priming in VV infection. VV, MCMV and *Listeria monocytogenes* infection induce high levels of IL-12, while LCMV infection is marked by an early and robust IFN-I response. We show, however, that the T helper dependence of CD8+ T cell priming after VV infection persists irrespective of experimental modulations of IFN-I or IL-12 signals.

P78

Differential activity patterns of IgG fractions, derived from intravenous immunoglobulin

Wymann S., Schaub A., Zürcher A.W., Stadler B.M., Miescher S.M.
University Institute of Immunology, DOLS, Inselspital, Bern, Switzerland

Depending on the formulation, intravenous immunoglobulin (IVIg) preparations contain variable amounts of monomeric and dimeric IgG. So far, little information is available on an eventual selective partition of Ab specificities into the monomeric and dimeric fractions nor their biologic significance. In serological assays, the dimeric fraction showed an increased recognition of a range of pathogen-associated protein antigens such as S. aureus Enterotoxin and Pseudomonas Exotoxin. Neutralisation assays were established for two selected target antigens; diphtheria toxin (DT) and Respiratory Syncytial Virus (RSV) to assess the functional significance of the differential serological reactivities. Measurement of the proliferation of DT-challenged cells showed equal neutralising capacities for both monomeric and dimeric fractions. Additionally, both IVIg fractions showed equal RSV-neutralising capacities. In contrast, IVIg fractions, analysed for anti-Pseudomonas LPS, anti-Pneumococcus polysaccharide or anti-H. influenzae B polysaccharide activity, showed a marked increased reactivity within the monomeric IVIg fraction. Comparison of dimer activity to that of monomerised dimers showed a reduced activity which may indicate blocking activity in the dimer fraction, assumed to represent antiidiotype activity which is thought to play a role in the immunoregulatory effects of IVIg. Glycoprofiles of Abs are important for differential IgG-Fc function. Ravetch et al showed in mouse models that IgG Fc fragments containing sialic acid residues demonstrated anti-inflammatory activity. However, no difference was seen between sial. IVIg or nonsial. IVIg in a comparative analysis of their pro-inflammatory potential using simulated immune complexes (IC) of polystyrene beads coated with either sial. IVIg or nonsial. IVIg. Both types of IC induced iMoDC maturation and activation to similar extents, shown by up-regulation of CD80, CD83 & CD86 as well as by the induction of pro-inflammatory cytokines (IL-8, TNFα, IL-6).

P79

HLA C allotypes differentialy support regulatory allo-specific NK cell-function

Zenhaeusern G., Gasser O., Hönger G., Durovic B., Stern M., Hess Ch.
University Hospital Basel, Department Biomedicine, Basel, Switzerland

Natural killer cells (NK cells) are a key element of the early innate immune response. Based on their expression of CD56 (neural cell adhesion molecule) and CD16 (FcγRIII), NK cells can be divided into CD56bright and CD16dim/negative NK cells. Whereas CD56dim NK cells are efficient killer cells, CD56bright NK cells contain low levels of perforin and granzymes, but, when activated by cytokines in vitro, can be induced to secrete large amounts of inflammatory and – to some extent – anti-inflammatory cytokines. NK cell-activity is triggered vis-à-vis cells lacking expression of autologous MHC I ("missing-self" recognition), whereas MHC I actively "silence" NK cell-activity. The same 'missing self' principle is thought to underlie NK cell allo-reactive killing. The dominant pattern of NK cell alloreactivity is due to recognition by the NK cell of two HLA C allotypes, determined by an asparagine/lysine polymorphism at position 80. Lack of recognition of these two HLA C epitopes vis-à-vis a given NK cell leads to a loss of inhibition of the corresponding NK cell, and hence to its allospecific activation. Unexpectedly therefore, we found that a subset of NK cells was activated rather than silenced when interacting with cells expressing normal levels of autologous MHC I. Instead of inducing an inflammatory phenotype, however, activation led to the secretion of the regulatory cytokines TGFβ and IL-10. Importantly, in vitro models of allogeneic interactions showed that co-expression of HLA C1 and C2 epitopes on target-cells best supported – or even enhanced – this cell-contact mediated regulatory NK cell-function. Together these data ascribe a novel pattern of reactivity to NK cells with potential implications both in autologous and allogeneic systems.

P80

Ephrin-A4 expression in CLL B-cells is linked to a reduced adhesion to endothelial cells

Alonso-Colmenar L.-M ,Trinidad E.M., Ballesteros M., Zapata A.G.
Complutense University of Madrid, Microscopy and Cytometry research center, Madrid, Spain

Chronic lymphocytic leukemia (CLL) of B-cells is characterized by a progressive accumulation of the malignant B-cell clone in the peripheral blood of patients leading to an state of chronic lymphocytosis. The study of the mechanisms governing CLL cells extravasation into lymph nodes, which is linked to a poor prognosis, is a central aspect of the pathophysiology of this disease, as interfering CLL cells dissemination might have therapeutical potential. We have previously reported the expression of several members of the Eph receptor tyrosinekinase family in CLL, largely implicated in cell adhesion processes in other systems. Overall, CLL B-cells showed higher expression levels of the ephrin A4 (EFNA4) member than normal B-cells and, interestingly, a lower expression among them was significantly associated with the occurrence of lymphoadenopathy. Besides, the EphA2 receptor for EFNA4 was highly expressed in the high endothelial venules of lymph nodes, suggesting that EphA2-EFNA4 interaction could be implicated in the extravasation of CLL B-cells. In vitro, CLL B-cells showed an impaired adhesion to HUVECs as well as transendothelial migration (TEM) capacity as compared to normal B-cells, while a differential response among CLL B-cell samples from patients with and without lymphadenopathy was observed, further relating with EFNA4 expression levels. EFNA4 signaling into B-cells, achieved through pre-treatment with soluble recombinant protein dimmers of the EphA2 receptor, largely reduced the number of cells adhered to extracellular matrix molecules like fibronectin, laminin or collagen as well as to cell adhesion molecules expressed by HUVECs like ICAM-1 or VCAM-1. In conclusion, the high expression of EFNA4 shown by CLL B-cells may be linked to an impaired extravasation capacity. Thus, managing EFNA4 signaling in CLL may represent a new therapeutic approach to prevent CLL cells dissemination during disease progression.

P81

Immunoglobulin E (IgE) and cytokine polymorphism in the pathogenesis of severe malaria in Ghanaian children

Amoako-Sakyi D., Asmah R.H., Kusi K.A., Troye-Blomberg M. Dodoo D., Gyan D.
University of Cape coast, school of Medical science Private mail bag 00233, Cape coast, Ghana

Introduction: The IL-4/Stat6 signalling pathway could be crucial for Th2 mediated immunity and protection against malaria. Although we and others have previously shown associations between some IL-4 polymorphisms and severe malaria, the role of Stat6 and IL-4Rα; polymorphisms in malaria pathogenesis is yet to be established. This study investigated the distinctive and interactive association of known polymorphisms of the IL-4 gene (+33C/T, 590C/T, VNTR), IL-4R gene (Arg551Gln) and STAT6 gene (1570C/T) with total IgE production and subsequently, malaria severity in Ghanaian children.
Methods: PCR-RFLP was used to genotype all polymorphisms in a hospital based cross-sectional study involving 290 malaria cases and controls. Malaria cases were categorized into uncomplicated malaria (UM), severe malarial anaemia (SMA), and cerebral malaria (CM).
Results: We found that a single nucleotide polymorphism (SNP) (rs3024974) which causes a C to T change in intron 18 of the stat6 gene is associated with protection from cerebral malaria (OR = 0.361, P = 0.0107). All other polymorphisms studied did not show any association with malaria severity except the IL-4 VNTR polymorphism. Our data did not show any association between rs3024974 and levels of total IgE.
Discussion: Data from this study suggests that rs3024974 is associated with protection against cerebral malaria in Ghanaian children. However, this protection maybe mediated by other factors other than total serum IgE. To the best of our knowledge, this study is the first to suggest a role for the stat6 SNP (rs3024974) in malaria pathogenesis.

P82

Are all donor-specific antibodies detected by solid phase assay before transplantation clinically relevant?

Aubert V., Venetz J.-P., Pantaleo G., Pascual M.
Service d'immunologie et d'allergie, Département de Médecine, CHUV, Lausanne, Switzerland

Since new technologies based on solid phase assays (SPA) have been routinely used in the transplant immunology laboratory, the presence of pre-transplant donor-specific antibodies (DSA) against HLA antigens has been considered as a risk factor for antibody-mediated rejection (AMR).To investigate the clinical relevance of pre-transplant DSA we screened renal transplant recipients for circulating anti-HLA antibody and DSA before transplantation.
Methods: 114 serum samples collected before transplantation were tested with highly sensitive and anti-HLA specific Luminex assay (LABScreen Single Antigen I and II, One Lambda, Inc., Canoga Park CA). Briefly, 20 ml of serum samples was added to 5 ml of beads, incubated in the dark for 30 minutes and then washed with buffer. 100 ml of goat anti-human IgG secondary antibody conjugated with R-phycoerythrin was added to the beads, incubated for 30 minutes in the dark, then washed and read on the LABScan 100. The cutoff value of the assay was 500 mean fluorescence index (MFI).
Results: Using this multiplex technology, 55/114 patients had anti-HLA antibody pre-transplant (48.2%) 11 of them had DSA (9.6%). From the 55 sensitized patients, 18 (16%) developed anti-HLA class I, 14 (12%) anti-HLA class II and 23 (20%) anti-HLA class I and II antibodies. Out of 11 biopsy-proven acute rejection episodes, 2 had DSA (18%), 3 (27%) had anti-HLA class I, 1 (9%) anti-HLA class II and 1 (9%) anti-HLA class I and II antibodies. Conversely, 9 from 11 transplant recipients had DSA without any post-transplant rejections episodes within 1 year after transplantation.
Conclusions: We found a relatively high prevalence of pre-transplant anti-HLA antibody by Luminex technology in our renal transplanted patients. But frequency of positive DSA before transplantation were around 10%. The deleterious effect of preexisting DSA on graft function were not observed in our study. Therefore, if we would have taken into consideration the presence of DSA as an absolute contra-indication to transplant, 8% would not have been transplanted.

P83

CpG based immunotherapy reverses BTLA mediated inhibition of human tumor antigen specific CD8 T cells

Derré L., Rivals J.-P., Jandus C., Romero P., Michielin O., Olive D., Speiser D.-E.
Ludwig Institute for Cancer Research, Division of Clinical Onco-Immunology / CHUV, Lausanne, Switzerland

Antigen specific CD8 T cells can protect from infectious and malignant disease. However, several inhibitory pathways compromise T cell expansion and function. The inhibitory receptors CTLA-4 and PD-1 may downregulate T cell responses against self and non-self antigens. Recently, B and T lymphocyte attenuator (BTLA) has been identified as a third inhibitory receptor that is frequently expressed by T cells. BTLA represses T cell proliferation and cytokine production upon binding to its ligand, herpes virus entry mediator (HVEM). HVEM is a member of the TNFR superfamily and is expressed mainly by T lymphocytes and APCs. Unlike CTLA-4 and PD-1, BTLA is constitutively expressed in naïve T cells. Here we show for the first time in humans, that BTLA is gradually downregulated during CD8 T cell differentiation from naïve to effector cells. Furthermore, melanoma cell lines were generally HVEM positive, correlating with frequent HVEM expression by melanoma cells in vivo. After vaccination of melanoma patients with Melan-A peptides, in vivo BTLA expression by Melan-A specific CD8 T cells was persistently high. As a result, these T cells remained susceptible to inhibition upon encounter with HVEM expressed by melanoma cells, as revealed by reduced IFNγ and TNFα production ex vivo. In contrast, when CpG oligonucleotides (PF-3512676) were added to the vaccine formulation, responding tumor antigen specific CD8 T cells progressively downregulated BTLA in vivo, down to similarly low levels as in CD8 T cells specific for influenza or herpes viruses. Consequently, T cells became resistant to HVEM mediated inhibition. These data provide an explanation for the strong immune responses by virus specific T cells, and tumor antigen specific T cells induced by CpG based vaccination. In conclusion, BTLA triggering represents a novel pathway of human T cell inhibition.

P84

Secretion of IFNγ vis-à-vis silenced donor-cells accurately identifies kidney transplant recipients at risk for immune-mediated injury

Durovic B., Zenhäusern G., Hönger G., Gasser O., Steiger J., Schaub S., Hess C.
Universitätsspital Basel, Departement Biomedizin, Basel, Switzerland

The ability to detect, and hence avoid, donor-specific antibodies (DSA) has drastically reduced – yet not eliminated – early allograft rejection rates. Huge efforts have been made to develop cellular assays identifying DSA-negative individuals at risk for rejection. However, lack of robustness and high and variable backgrounds have limited usefulness of these assays, and hindered their transition into clinical routine. Here we developed a novel ELISpot-based assay designed to detect allo-specific IFNγ-secretion against a close-to-zero background. The experimental system was optimized using HLA-typed allo-specific cell-lines in classic killing- vs. ELISpot-assays. The clinical performance of the test-system was then examined in a prospective cohort of 38 kidney transplant recipients, all undergoing protocol-biopsies at 3 and 6 mos post-transplantation. Peripheral blood mononuclear cells (PBMC) collected prior to transplantation, and at 3, 6 and 12 mos post-transplantation were assayed against organ-donor

derived cells. The specificity of reactivity was tested in third-party experiments. In recipients without evidence of rejection (n = 18), no donor-specific IFNγ production was observed. By contrast, in 6 of 20 patients with biopsy-proven rejection (clinical or sub-clinical), donor-specific IFNγ production was detected (rej. [6/20] vs. no rej. [0/18], p = 0.02 [Fisher's exact test], sensitivity 30%, specificity 100%). In none of these 6 patients DSA were present before transplantation. PBMC from 5/6 individuals were available for testing against HLA-unrelated third-party targets (n = 2 each). In only 1 of 10 experiments secretion of IFNγ was induced. Importantly, *on each occasion* IFNγ secretion was detected *prior to clinical and/or simultaneously to sub-clinical* rejection episodes. Together these data establish this assay as (i) highly specific –largely eliminating cut-off issues and (ii) sensitive to identify a DSA-negative segment of kidney transplant recipients at risk for allo-specific graft-injury. Prospective studies will need to establish the potential role of this assay in the clinical management of kidney transplant recipients.

P85

Total CD4+ T cell-counts loose surrogate-character for the CMV-specific CD8+ T cell-response upon initiation of iatrogenic immunosuppression

Gasser O., Zenhäusern G., Hönger G., Brander C., Hess C.
University Hospital Basel, Department Biomedicine, Immunobiology Lab, Basel, Switzerland

Enumerating total circulating CD4+ "helper" T cells as surrogate-marker for the functioning of CD8+ T cell-mediated immunity is common practice. However, little is known on how – before and after initiation of iatrogenic immunosuppression – total CD4+ T cell-counts actually relate to (i) the frequency of virus-specific CD4+ T cells, and (ii) the breadth and magnitude of CD8+ T cells targeting the same virus.
Patients and methods: Here we longitudinally monitored total and CMV-specific CD4+ T cells in a cohort of 33 CMV-aviremic kidney-transplant recipients. CD4+ T cells were quantified using a virus-lysate based ELISpot assay. In parallel, we assessed the breadth and magnitude of CD8+ T cell-responses specific for a set of 38 CMV-derived epitopes.
Results: Pre-transplantation, total and CMV-specific CD4+ T cell-numbers correlated with both breadth and magnitude of the CMV-specific CD8+ T cell-response. While this correlation was retained under immunosuppressive therapy in the case of CMV-specific CD4+ T cells (week 52 post-transplantation: r = 0.7727, p = 0.014 and r = 0.7704, p = 0.015 for magnitude and breadth, respectively), the association of total CD4+ T cell-counts and CMV-specific CD8+ T cell-immunity was lost post-transplantation (week 52 post-transplantation: r = 0.0243, p = 0.94 and r = –0.0152, p = 0.62 for magnitude and breadth, respectively).
Conclusion: Our results indicate that – in the pharmacologically immunosuppressed host – total CD4+ T cell-counts need to be interpreted cautiously when used to derive estimates regarding CD8+ T cell-mediated – i.e. cytotoxic – immunity. How well virus-specific CD4+ T cells predict immune-protection conferred by these cytotoxic CD8+ T cells now needs to be established.

P86

Chronic idiopathic urticaria: detection of autoantibodies against FcεRI and IgE by CD63 and CD203c upregulation on basophils

Gentinetta Th., Hausmann O., Keller M., Pichler W.J.
ADR-AC GmbH, Adverse Drug Reactions – Analysis and Consulting, Bern Switzerland

Introduction: Chronic idiopathic urticaria (CIU) is defined by the occurrence of pruritic wheals for at least 6 weeks. A subset of patients (35–50%) with CIU seems to have autoantibodies against the IgE receptor (FcεRI) or against cell-bound IgE on mast cells and basophils, which could lead to degranulation and the typical symptoms. They can be detected by the autologous serum skin test (ASST), or by incubating patient serum with basophils and measuring their activation/degranulation in vitro. To replace the ASST, we established the basophil activation test (BAT) for CIU.
Methods: Sera of 39 patients suffering from CIU (16 ASST+, 9 ASST-, 14 ASST not done) were tested by incubating serum with well defined basophils from healthy donors. The ability of the serum to induce upregulation of CD63 and CD203c was measured by flow cytometry using a three color strategy with anti-CD3, anti-CCR3 and anti-CD63/CD203c. CD63+ (reflecting degranulation) or CD203c+ (reflecting activation) on basophils were identified on CCR3+/CD3- cells. 11 healthy donors were included to determine the optimal cut-off point.
Results: The upregulation of CD63 was more specific and sensitive than upregulation of CD203c, and is correlated to the ASST (R = 0.42). In 7/25 donors, discrepant results were observed, as 1 serum was positive in ASST but negative in BAT and 6 positive in BAT but negative in ASST. Monitoring over time revealed that disappearance of symptoms were accompanied by loss of BAT reactivity.
Conclusion: IgG autoantibodies against FcεRI or against cell-bound IgE can be detected by measuring upregulation of CD63 or CD203c in vitro. This test can replace the ASST. Its advantages are a 1) better safety; 2) it detects the autoantibody and its activity; 3) it provides a more quantitative measurement; and 4) the BAT in CIU is well suitable to monitor the course of the disease.

P87

Progression of cutaneous squamous cell carcinoma in immunosuppressed patients is associated with reduced CD123+ and FOXP3+ cells in the perineoplastic inflammatory infiltrate

Hofbauer G., Mühleisen B., Petrov I., Gächter T., Kurrer M., Schärer L., Dummer R., French L.E.
University Hospital Zurich, Dermatology, Zurich, Switzerland

Squamous cell carcinoma of the skin (SCC) increases dramatically in organ transplant recipients (OTRs). We assumed that qualitative and quantitative differences in perineoplastic inflammation in OTRs contribute to the increased carcinogenesis.
Material and methods: We studied perineoplastic SCC inflammatory infiltrate assessing depth, density and phenotype (CD3, 4, 8, FOXP3, CD123, and STAT1) by immunohistochemistry in paired biopsies of intraepithelial and invasive SCC in immunocompetent patients and OTRs.
Results: Considerable inflammation was observed in all intraepithelial SCC (inflammatory infiltrate depth 2.80 mm ± 2.21 immunocompetent pts, 2.15 mm ± 2.95 OTRs). Inflammation was more pronounced in invasive SCC of immunocompetent patients (4.60 ± 4.67 mm) and OTRs (3.30 ± 5.90 mm) respectively (p <0.005). The density of perineoplastic inflammatory infiltrates increased from intraepithelial to invasive SCC (p = 0.005). OTRs show a lower density of perineoplastic inflammatory infiltrate (p = 0.041). OTRs also show reduced CD3+ T-lymphocyte and CD8+ cytotoxic T-lymphocyte proportions in intraepithelial SCC (p = 0.025 and 0.027, respectively). FOXP3+ regulatory T-lymphocyte proportions in OTRs' invasive SCC are markedly diminished (p = 0.048). CD123+ plasmacytoid dendritic cells increase in the progression from intraepithelial to invasive SCC in immunocompetent patients (p = 0.040). CD123+ cells are reduced in all SCC of OTRs (p = 0.036).
Conclusions: Perineoplastic inflammation in intraepithelial SCC is pronounced both in immunocompetent patients and OTRs. Inflammation increases further in invasive SCC. OTRs show reduced proportions of FOXP3+ regulatory T cells and CD123+ plasmacytoid dendritic cells. This distinct inflammatory infiltrate may result in the increased cutaneous carcinogenesis and more aggressive behaviour of SCC in OTRs.

P88

Multiple sclerosis and polyangitis: is there any correlation?

Jandus P., Györik S., C. Marone
Ospedale Regionale di Bellinzona e Valli, Dipartimento di Medicina Interna, Bellinzona, Switzerland

A 75 years old woman presented with rectorragia and pain in the right lower abdominal quadrant, accompanied by nausea and vomiting. For one month before admission she was suffering of a non productive cough with intermittent fever reaching 38 °C. Her medical history is known for a 15 years old history of multiple sclerosis stable on interferon therapy. Clinical examination showed pain on palpation in the right lower abdominal quadrant with defense. The reminder examination was normal. Analysis revealed normal full blood count and normal serum biochemistry, but an elevated C- reactive protein 160 mg/l (<5 mg/l). A colonoscopy disclosed acute erosive colitis. Chest radiography showed multiple nodulary opacities. CT scan of the chest confirmed infiltrative nodules and cavitary lesions. Transbronchial biopsies and lavage were not contributive, showing only inflammatory signs compatible with bronchopneumonia. Microbiological analyses were negative. The urine analysis was pathologic with erythrocyturia, leucocyturia, hyaline and granulocytic casts. The antibody study was positive for ANCA-MPO. A renal biopsy depicted focal and segmental necrotizing glomerulonephritis with beginning crescent formation associated with arteriolar lesions. The diagnosis was then microscopic polyangitis with ANCA positive glomerulonephritis, gastro-intestinal and pulmonary lesions. A therapy with high dosed Steroids and Cyclophosphamide 2 mg/bw/day was then started with sudden and progressive improvement of the clinical situation. In the past, case report studies suggested an association between multiple sclerosis and other autoimmune diseases. However, there were sources of selection and reporting bias. A new large Canadian cohort study did not record an increased frequency of common autoimmune diseases in multiple sclerosis patients compared with spousal controls. Interestingly abnormally high frequency of circulating antibodies (6.6% ANCA and 33.3% ANA) has been reported in patients with multiple sclerosis. Whether these autoantibodies have got any clinical significance or represent an epiphenomenon is still unknown.

P89

Cytokine MRNA profile of EBV- and CMV-specific T cells in multiple sclerosis

Jaquiéry E., Jilek S., Schluep M., Garcia M., Pantaleo G., Du Pasquier R.A.
Centre Hospitalier Universitaire Vaudois, Division of Immunology and Allergy, Lausanne, Switzerland

We have previously shown that there was an enrichment in highly differentiated (CCR7-) CD8+ T cells in the cerebrospinal fluid of patients with early multiple sclerosis (MS). We have also found that the same category of patients had a high Epstein-Barr virus (EBV)-specific, but not an increased cytomegalovirus (CMV)-specific, CD8+ T cell response in the blood, suggesting that EBV might be involved in the pathogenesis of MS. We decided to explore whether this increased EBV-specific CD8+ T cell response in early MS patients was related to a dysregulated cellular immune response against this virus. To this purpose, we studied the mRNA expression of different cytokines that are considered to be relevant in MS after EBV or CMV stimulation of T cells in MS patients and healthy controls (HC). T cells were discriminated between naïve and central memory (CCR7+) on one hand, and effector memory and effector T cells, thus highly differentiated T cells (CCR7-), on the other hand. Except for an increased IFNγ mRNA expression by EBV-stimulated highly differentiated (CCR7-) CD8+ T cells, we did not find a different cytokine mRNA expression pattern between MS and HC after EBV stimulation, suggesting that the increased frequency of EBV-specific CD8+ T cells found in the blood of MS patients is not linked with a dysregulated T cell response against EBV. This study was supported by grants from the Swiss National Foundation and by the Swiss MS Society.

P90

Platelet derived-ectosomes have anti-inflammatory properties

Sadallah S., Eken C., Martin P., Schifferli J.A.
University Hospital Basel – Biomedicine Dept., Immunonephrology Lab., Basel, Switzerland

Many eukaryotic cells release vesicles spontaneously or under appropriate stimulation. Beside the release of preformed vesicles, many cells shed small membrane vesicles, which are budding directly from the cell membrane and thus called ectosomes. Stored human platelets for transfusion contain “microparticles” formed by ectocytosis. The number of such ectosomes released by platelets (Plt-Ect) increases over time, so that large quantities of them are transfused together with platelets in patients. It is well known that Plt-Ect promote haemostasis and many publication emphasize their proinflammatory activities on endothelial cells. Here, we isolated and analysed Plt-Ect released during platelet storage. By EM Plt-Ect size ranged from 200 to 850 nm. By FACS they were CD61+ and the preparations were no contaminated with leukocyte-derived particles (no CD45). As expected factor H of complement was found on the surface of Plt-Ect. The binding of annexinV indicated high expression of phosphatidylserine (PS). The release of TNF and IL10 by macrophages when exposed to zymosan was inhibited by Plt-Ect in a dose dependent manner. Interestingly the pre-exposure of the macrophages to Plt-Ect rendered them insensitive to later exposure to zymosan, even when the pre-exposure occurred 24 h before, suggesting that Plt-Ect induce a reprogramming of macrophages. The effects of Plt-Ect could be blocked by pre-incubating them with annexinV suggesting that PS was a major player in the anti-inflammatory properties of Plt-Ect. The same inhibition of TNFα, IL10 as well as IL6 release was seen when immature dendritic cells (DCs) were activated by LPS in the presence of Plt-Ect. Finally, the Plt-Ect interfered with the in vitro differentiation of monocytes towards immature DCs by GMCSF/IL4. In sum, these data indicate that Plt-Ect have inhibitory effects on macrophage/DC activation and thus appear to be more modulators of the inflammation in thrombosis than just “proinflammatory”.

P91

Determination of IL-5 in serum by a highly sensitive flow-cytometry based assay: presentation of the method and first clinical data

Urwyler A., Pichler W.J.
Cytolab, Lab, Dällikon, Switzerland

Determination of cytokine values in serum have not yet found entrance into clinical diagnosis, as the relevance of such determinations, and in particular the possibility to detect minute amounts of cytokines in the serum, were not established. On the other hand, the determination of IL-5 in serum has been recommended (1) as one of the first steps to differentiate between intrinsic and extrinsic forms of eosinophilia, as IL-5 is mainly produced by T-cells, and its enhanced presence in the serum would favor a T-cell mediated mechanism.
Methods: We established a optimised flow-cytometry based assay using a human IL-5 MAP Fluorokine Kit from R&D Systems. Analysis were done on a conventional flowcytometer (FC 500 MPL from BeckmanCoulter). We collected serum of patients with different degree of eosinophilia, and compared the eosinophil count, level of ECP and IL-5 values.
Results: The assay proved to be highly reproducible and sensitive in the low picogramm (pg) range (Detection Limit: 0.15 pg/ml). Normal, healthy persons with eosinophil counts below 0,4G/l have values below 0.15 pg/ml; marginally elevated eosinophil counts go along with IL-5 values between 0.15 to 0.5 pg/ml. Higher values of eosinophils have IL-5 levels above 0.5–3 pg/ml. Consequently, serum IL-5 levels correlate well to eosinophil counts (R = 0.96) and ECP levels (R = 0.84). The highest value observed stems from a patient with massive eosinophilia (5.01 G/l, normal <0.4 G/l, IL-5 = 5.63 pg/ml), which was diagnosed as chronic eosinophilic leukemia due to FIP1L1-PDGFRα; fusion-transcripts.
Conclusions: IL-5 values can be measured in the serum by a simple, robust and very sensitive technique. Further work is needed to evaluate whether IL-5 levels can be used to differentiate between intrinsic and extrinsic, mostly T cell regulated eosinophilic disorders (1). However, it is sure that IL-5 determinations will provide interesting informations with regard to the pathogenesis of diseases with hypereosinophilia.
Simon D & Simon HU, Eosinophilic disorders, JACI 2007; 119:1291-1300

P92

The recipient killer cell immunoglobulin-like receptors (KIR) and their donor HLA ligands influence the graft function after kidney transplantation

Villard J., de Rham C., Ferrari-Lacraz S., Hadaya K., Bandelier C., Tiercy J.-M., Martin P.-Y.
Geneva University Hospital, Immunology and Transplant Unit, service of Immuno, Geneva, Switzerland

The role of natural killer cells (NK) and their receptors are emerging after solid organ transplantation (SOT). Not targeted by the immunosuppressive therapy given to prevent rejection, recent data suggested that NK cells could play a critical role in the anti-CMV immunity and in the risk of rejection or graft function. NK cell activity is mediated by different receptors, among them, the KIR family is of special interest. The KIR family is composed of activating and inhibitory receptors which bind mainly to HLA-C or Bw4 ligands. According to the theory, three major genetics factors could determine the degree of NK cell reactivity after SOT: the KIR genes content of the recipient, the HLA-C and Bw of the recipient and the HLA-C and Bw allotype of the donor. In this preliminary study, 68 patients after kidney transplantation were analysed for their KIR and HLA genes and all donors were typed for HLA-C and Bw. The recipients were stratified in four groups according to the presence of inhibitory or activating KIR (haplotype A or B respectively) and the presence or absence (missing ligands) of allogenic (donor) HLA ligands. The four groups were analysed with regards to CMV reactivation, rejection and graft function at 1 year. We confirmed our previous data with regard to the role of activating KIR and the importance of missing HLA ligands of the recipient and the risk of CMV reactivation in the first 6 months after transplantation. Donor allogenic HLA-C or Bw ligands have no influence on the rate of CMV reactivation. Interestingly, we also demonstrate that in the group of recipients characterizes by a predominance of KIR inhibitors (haplotype A) and the presence of their HLA ligands in the graft, the graft function is worse compared to the other groupsat 12 months. Our data suggest that recipient KIR inhibitors and the presence of their cognate HLA ligands in the donor graft, resulting in an inhibition of NK cell function, could be directly or indirectly of great importance to predict graft function at one year after kidney transplantation.

P93

High levels of Anti-apolipoprotein A-1 IgG are associated with 1-year cardiovascular complications, higher matrix-metalloproteinase (MMP)-9, and lower MMP-3 levels in patients with myocardial infarction

Vuilleumier N., Pagano S., Charbonney E., Nkoulou R., Alvarez M., James R., Perneger Th., Roux-Lombard P.
Laboratory Medicine Service, Department of Genetics and Laboratory Medicine, Geneva University Hospitals and University of Geneva, Switzerland.

Apolipoprotein A-1 (Apo A-1) is the major protein fraction of high-density lipoproteins whose protective role in the cardiovascular system has been established. Anti-apoA-1 IgG are elevated in a significant subset of patients with myocardial infarction (MI) and seem correlated to oxidized Low Density Lipoprotein, a major player in atherogenesis and atherothrombosis.
Aim: to explore their relation with cardiovascular outcome and matrix-metalloproteinase (MMP) 3 and 9 – two emergent markers of atherosclerotic plaque vulnerability – in MI patients.
Methods: 221 consecutive MI patients who all underwent 12 months follow-up were included in this prospective study. The predetermined composite cardiovascular endpoint consisted in presence of death,

acute coronary syndrome, stroke or acute heart failure requiring hospitalization. Auto-antibodies were determined by Elisa and MMPs by multiplex technology.
Results: Anti-apoA-1 IgG were positive in 10% of MI patients. The composite endpoint was met by 13% of patients. MI patients with complications during the 1-year follow-up had higher anti-apoA-1 IgG values at baseline than patients without (median Index: 30.7 vs 20.6; p = 0.007). After adjustment for age, sex, diabetes, smoking, obesity and dyslipidemia, logistic regression analysis showed that anti-apoA-1 IgG positivity was associated to a 5-fold increase (Odds ratio: 5.12; 95%CI:1.8–14.9; p = 0.0004) of cardiovascular complications at one year. Furthermore, positive anti-apoA-1 IgG MI patients had higher MMP-9 (median: 631 vs 431 ng/ml; p = 0.01), but lower MMP-3 values (median: 9.7 vs 15.6 ng/ml; p = 0.01) than patients tested negative for those antibodies. Ranked Spearman test showed weak but significant correlations between anti-apoA-1 IgG and MMP-9 (r = 0.14; p <0.05) and MMP-3 (r = –0.16; p <0.05).
Conclusions: In MI, anti-apoA-1 IgG positivity appears to be an independent predictor of 1-year cardiovascular complications, associated to a vulnerable plaque promoting MMP profile. The causal nature of those associations remains to be determined.

P94

Muckle Wells syndrome in a child with unusual mutation and successfully treated by anakinra

Wassenberg J., Hofer M.
CHUV, Pediatrics, Division of Allergology and Immunology, Lausanne, Switzerland

The Muckle Wells syndrome, belonging to the cryopyrin-associated periodic syndromes (CAPS) is generally characterized by progressive sensorineural hearing loss, fever and cold-induced urticaria. It is commonly linked to mutations in the gene NLRP3 on chromosome 1.
Case-report: A 2-years-old boy known since his first year of life for a severe bilateral sensorineural hearing loss, fever, urticaria-like rash twice a month and occasional ankle arthralgia, showed a high elevated sedimentation rate. The laboratory work-up could exclude a chronic infection, immunodeficiency, auto-immunity and allergies. Hearing loss linked syndromes like Alport, Pendred and connexine 26-linked syndromes could be excluded. Q703K mutation on gene NLRP3 was found and based on the symptoms and the genetic analysis, a diagnosis of Muckle Wells syndrome was confirmed. The patient started a daily subcutaneous treatment of Anakinra, a recombinant selective interleukin-1 receptor antagonist, which induced significant improvement on fever, rash and arthralgia within 3 months of treatment.
Conclusion: In the presence of hearing loss, urticaria and fever in a child, it is important to look for CAPS. Mutation Q703K on the gene NLRP3 is found in 3% of the general population and has been reported to be associated with atypical neurological cryopyrin-associated periodic syndromes. We report here the first case of classical Muckle Wells syndrome with this mutation, associated with early-onset hearing loss. Our patient could be successfully treated by anakinra.

P95

Characterization of dendritic cells in irritant contact dermatitis

Yawalkar N., Tang H., Hassan A.S., Simon D.
Department of Dermatology, Inselspital, University of Bern, Bern, Switzerland

Irritant contact dermatitis is considered to be a non-allergic inflammatory reaction of the skin caused by direct cytotoxic effects of irritant chemical or physical agents. Although immunological mechanisms may be involved in these reactions, the role of dendritic cells in irritant contact dermatitis is poorly understood. In this study we sought to investigate the phenotype and distribution of dendritic cells in irritant patch test reactions (n = 13) in comparison to normal skin from healthy controls (n = 13). Skin biopsy specimens were obtained from patch test reactions induced by sodium lauryl sulfate (SLS). Normal skin samples were taken from age and sex-matched healthy subjects undergoing abdominal surgery and mammary reconstruction. Immunohistochemistry was performed with monoclonal antibodies against CD1a, CD1c, CD11c, CD68, CD83, CD206/mannose receptor, CD123, CD207/langerin, DC-LAMP/CD208, CD209/DC-SIGN, BDCA-2/CD303 and HLA-DR. Our results revealed a significant increase of different DC subsets including immature DC, mature DC as well as myeloid and plasmacytoid DC in irritant contact dermatitis in comparison to normal skin. This comprehensive characterization of the DC subpopulation provides novel data regarding the involvement of various DC subsets in irritant contact dermatitis and sheds more light on the role of the innate immune system in this type of reactions.

Posters – Host responses to infections

P96

Role of IL-7 in shaping the immune response to *Leishmania major*

Auderset F., Schmutz S., Allenbach C., Acha-Orbea H., Finke D., Tacchini-Cottier F.
WHO-IRTC, Department of Biochemistry, Université de Lausanne, Epalinges, Switzerland

The role of IL-7 in the development of infection and its associated immune response was investigated in the murine model of infection with *Leishmania major*. IL-7 has several properties favoring parasite elimination in infected macrophages as well as inducing the development of B cell, a cell population reported to present *Leishmania* antigens and influence T helper differentiation. To investigate the role of IL-7 in the protective immune response to *Leishmania major*, transgenic mice on a L. major genetic resistant background, overexpressing IL-7 systemically (H-IL-7Tg), were infected with the parasite, and the development of lesion and immune response were measured and compared to those developing in control mice. *L. major* infected littermate control mice were able to heal their lesions, eradicate their intralesional parasites and develop a protective Th1 immune response. In contrast, H-IL-7Tg mice developed unhealing lesions and failed to control their parasite burden despite secretion of significant levels of IFNγ. A small but significant increase in IL-4 was measured in T cells derived from draining lymph node of H-7Tg mice, with a corresponding increase in serum IgG1 and IgE. A significant increase in B cells was measured in spleen and draining LN of Tg mice, suggesting that this cell population together with IL-7 itself could be involved in the switch to a susceptible phenotype observed in the Tg mice. Thus, IL-7 has a dramatic effect on the susceptibility to *Leishmania major* infection, allowing the development of Th2 cells with a deleterious effect on the control of infection.

P97

Infected hepatocytes present *Plasmodium*-sporozoite antigens and induce protective immune response in mice

Balam S., Romero J.F., Bongfen S.E., Corradin G.
University of Lausanne, Department of Biochemistry, Epalinges, Lausanne, Switzerland

One target of protective immunity against liver stage of malaria in BALB/c mice is directed against the circumsporozoite protein (CSP) and predominantly involves IFNγ production by specific CD8+ T cells, which prevent development of exo-erythrocytic stages and subsequent blood infection. In our recent studies we showed that sporozoite-derived CS peptides are presented on the surface of infected hepatocytes in the context of MHC class I molecules and activate a CS specific CD8+ T cell clone. This indicates that primary hepatocytes can stimulate primed T cells. However, no clear data are available whether or not sporozoite infected hepatocytes are capable of priming naïve T cells in vivo. For this reason, we have established an in vivo protocol to address this question. Data will be presented which show that hepatocytes can indeed act as antigen presenting cells in primary CS specific T cell responses in vivo. Knowledge of the antigen processing and presentation capacity and requirements by infected hepatocytes is important in the elucidation of the steps leading to T cell priming during in vivo infection and may contribute in the design and development of pre-erythrocytic malaria vaccines.

P98

Limited innate immune response to LCMV infection is associated with weak CD8 and CD4 T cell expansion in infant mice

Belnoue E., Fontannaz P., Sealy G., Rochat A.-F., Lambert P.-H., Pinschewer D.-D., Siegrist C.-A.
University of Geneva, Pathology-Immunology, Geneva 4, Switzerland

Early life is characterized by an increased vulnerability to infectious agents, and in particular to viral pathogens. Cytopathic viruses such as influenza virus that induce acute infections in immunologically mature hosts, follow a protracted course in early life, characterized by higher viral loads and several weeks of viral replication. To identify the mechanisms responsible for this protracted pattern of infection, we

developed an infant infection model in 2-week-old BALB/c mice using the well-characterized LCMV-WE strain. We previously showed that LCMV-specific CD8+ T cells were elicited in infant mice but failed to expand and rapidly control infection. Here, we show that LCMV infection induces a lower number of multifunctional CD4+ T cells in infant compared to adult mice. Adoptive transfer of naïve adult CD4+ T cells did neither restore LCMV-specific CD8+ T cell responses nor the course of infection in infant mice. This suggested that the early life innate responses were not sufficient for optimal activation/induction of CD4+/CD8+ T cells during LCMV infection. Indeed, LCMV infection of infant mice fails to elicit an adult-like increase / activation of plasmacytoid dendritic cells, resulting into lower production of type I interferon. Strategies to increase type I IFN production and restore effective CD8/CD4 T cell responses in infant mice are being assessed.

P99

An essential role for neutrophil-secreted CCL3 in the early recruitment of dendritic cells to the site of *Leishmania* infection

Charmoy M., Brunner-Agten S. , Aebisher D., Launois P., Milon G., Proudfoot A., Tacchini-Cottier F.
WHO-IRTC, Department of Biochemistry, University of Lausannne, Epalinges, Switzerland

Neutrophils are rapidly and massively recruited to sites of infection. Here, their role in the early recruitment of dendritic cells (DCs) in response to infection was investigated using *Leishmania major* as infecting pathogen. Three chemokines, CCL3, CCL4 and CCL5, were shown to be transcribed and secreted by C57BL/6 mouse neutrophils exposed to L. major promastigotes. Chemokine presence correlated with the chemotaxis of immature DCs towards supernatants of L. major-loaded neutrophils. CCL3 was the neutrophil-secreted chemokine most induced in response to infection, and the DC chemo-attracting activity of neutrophil supernatant was markedly impaired once depleted of CCL3. One day post L. major inoculation, DCs migrated out of ear skin explants, a process being markedly decreased in mice depleted of neutrophils prior to infection. While no significant defect of neutrophil emigration was noticed in CCL3-/- mice or in mice given Evasin1, a CCL3 blocking protein, a significant defect in DC recruitment was detected at the site of parasite inoculation one day post infection, which was corrected by the injection of C57BL/6 neutrophils at the time of infection. These findings reveal the essential role of neutrophil-secreted CCL3 in the first wave of DC emigration to sites of infection.

P100

E7-specific immune responses induced in the genital mucosa of mice by novel immunotherapy against HPV-16 and cervical cancer

Decrausaz L., Goncalves A.-R., Bobst M., Nardelli-Haefliger D.
Institute of Microbiology, CHUV, Lausanne, Switzerland

Background: Infection with high risk Human Papillomavirus (HPV) is the necessary cause of cervical cancer. Therapeutic vaccines may eliminate lesions in already infected women who have no benefit from the recently available prophylactic vaccines. However, until now, therapeutic vaccines only showed limited clinical results, possibly linked to an inefficient targeting of protective immune responses to the genital mucosa together with an associated basic immunosuppressive environment.
Objectives: Develop novel immunotherapies that effectively enhance the vaccine-specific immune responses in the genital mucosa.
Methods: Mice were subcutaneously immunized with an adjuvanted synthetic HPV16 E71-98 polypeptide vaccine. Tumor regression was evaluated using TC-1 cells. Antibody-mediated in vivo depletion of CD4 T, CD8 T or NK -cells were performed during tumor protection assays. E7-specific CD8 T cell effector responses were determined by ex-vivo IFNy ELISPOT and in vivo cytotoxic assays. Intravaginal administration of immunostimulants (Toll-like receptors agonists) was used in combination with vaccination.
Results: Immunization with E7 vaccine induced 100% regression of subcutaneous tumors located in the flank of mice, in both prophylactic and therapeutic settings. In vivo cell-depletions demonstrated that tumor regression was essentially mediated by CD8 T cells. Indeed, E7-specific CD8 effector cells were detected in the blood, different lymphoid organs and more importantly in the genital mucosa it-self. There was no correlation between the responses measured in the periphery with those measured in the genital mucosa of individual mice. The additive topical application of different TLR agonists greatly enhanced the E7-specific responses locally in the genital mucosa. Finally, preliminary data showed that our immunotherapeutic strategies could induce protection against vaginal TC-1 tumors.
Conclusion: Our data highlight the necessity to determine the immune responses directly in the genital mucosa and suggest that combination of an adjuvanted E71-98 peptide with topical immunostimlants could be an efficient immunotherapy against HPV-16 and cervical cancer.

P101

Innate immune sensing of modified vaccinia virus ankara (MVA) is mediated by cross-activation of TLR2-TLR6, MDA-5 and the NALP3 inflammasome

Delaloye J., Roger T., Steiner-Tardivel Q.-G., Le Roy D., Tschopp J., Pantaleo G., Esteban M., Calandra T.
CHUV, Infectious Diseases, BH 19-111, Lausanne, Switzerland

MVA is an attenuated dsDNA poxvirus currently developed as a vaccine vector against HIV/AIDS. Profiling of the innate immune responses induced by MVA is essential for the design of vaccine vectors and for anticipating potential adverse interactions between naturally acquired and vaccine-induced immune responses.
Methods: Mouse BMDMs and human PBMCs, THP-1 cells and THP-1 cells transduced with shRNA against TLR2, IPS-1, NALP3, Caspase-1 or ASC were infected with MVA. Cytokine and chemokine mRNA and protein were quantified by real-time PCR, ELISA and Luminex technology. The activation of transcription factors was analyzed by EMSA, Western blotting and transient transfection.
Results: The innate immune responses elicited by MVA were characterized by a robust chemokine production and a weak pro-inflammatory cytokine response. Analyses of cytokine production by BMDMs isolated from mice deficient in TLRs, MyD88 and TRIF revealed a critical role for TLR2, TLR6 and MyD88 in the production of IFNβ-independent chemokines. MVA markedly up-regulated the expression of RIG-I, MDA-5 and IPS-1. Reduced expression of RIG-I, MDA-5 and IPS-1 by shRNAs indicated that sensing of MVA by RLR and production of IFNβ and IFNβ-dependent chemokines was controlled by the MDA-5 and IPS-1 pathway. Crosstalk between TLR2-MyD88 and the NALP3 inflammasome was essential for expression and processing of IL-1β. Transcription of the IL-1β gene was impaired in TLR2-/- and MyD88-/- BMDM, whereas mature and secreted IL-1β was massively reduced in NALP3-/- BMDM and in human THP-1 macrophages with reduced expression of NALP3, ASC or caspase-1.
Conclusion: Innate immune sensing of MVA and production of chemokines, IFNβ and IL-1β by macrophages is mediated by the TLR2-TLR6-MyD88, MDA-5-IPS-1 and NALP3 inflammasome pathways. Delineation of the host response induced by MVA is critical for improving our understanding of poxvirus antiviral escape mechanisms and for designing new MVA vaccine vectors with improved immunogenicity.
Research supported by the Bill & Melinda Gates and the Swiss National Science Foundation.

P102

A novel in vivo model of infection with *Leishmania guyanensis* parasites

Ives A., Ronet C., Prevel F., Breton-Revez M., Launois P., Fasel N., Masina S.
Department of Biochemistry, UNIL, Epalinges, Switzerland

Mucocutaneous leishmaniasis (MCL) is associated with parasites of the *Leishmania Viannia* subgenus, including *L. guyanensis*. This disease is characterized by intense activation of inflammatory cells and extensive tissue destruction including the degradation of cartilage. The lack of information on the host immune responses following infection with *L. guyanensis* prompted us to investigate the behavior of this species in vivo in mice. We infected BALB/c mice with either *L. guyanensis* M5313 parasites (metastatic in a hamster model), MCL patient parasite isolates, cutaneous leishmaniasis patient isolates, or *L. major* LV39 parasites to serve as a control. The M5313, or MCL parasites showed a reproducible susceptible phenotype in at least 50% of infected mice characterized by the development of non-healing, non-necrosing lesions that persisted with high parasitemia over 13 weeks post infection which was associated with the development of a Th2 immune response. The susceptibility of these BALB/c mice depended both on IL-10 and IL-4, as IL-10$^{-/-}$ on a BALB/c background, and BALB/c mice treated with anti-IL4 were able to control infection with M5313 parasites. C57BL/6 mice infected with M5313 were highly resistant to infection and produced transient footpad swelling that healed by week 9 post-infection, with low degrees of footpad parasitemia, and a Th1 polarized immune response. Infection of mice deficient in MyD88, TRIF, TLR3, and TLR9 (on a C57BL/6 background) indicated that MyD88 and TLR9 were involved in the resistance to infection with M5313 parasites, and that TRIF and TLR3 were involved in the susceptibility. This is the first report describing a potential murine model of *L. guyanensis* infection and these findings could help to increase the biological understanding of the immune response caused by *Leishmania* parasites leading to MCL in humans which in turn provides insights for new MCL therapies.

P103

Identification of PD-1 as a unique marker for discordant immune response in HIV infected patients

Pfistershammer K., Rieger A., Steinberger P., Kohrgruber N.
Medical University Vienna, Department of Dermatology, Wien, Austria

Objectives: PD-1 expression on T cells correlates with T cell exhaustion and disease progression in HIV infected patients. HAART results in viral suppression and in reduced PD-1 expression and immune restoration in most patients. However, a minority of patients fails to reconstitute their CD4 T cells in spite of successful suppression of HIV replication.
Design: In this study we assessed PD-1 expression on T lymphocytes in patients with failing immune recovery.
Methods: PD-1 expression was analyzed by FACS on CD4 and CD8 T cells of HIV infected patients showing a defect in immune reconstitution following HAART as defined by less than 300 CD4 cells/mm³ in spite of viral suppression and compared to PD-1 levels on T cells of patients with good immunological recovery.
Results: We found persistence of high PD-1 expression on CD4 and CD8 T cells in spite of viral suppression in patients with poor immune reconstitution. In contrast failing immune reconstitution was not associated with the expression of other markers associated with immune activation or enhanced endotoxin serum levels. Furthermore we show that these T cells differ from T cells of an aged immune system. We find that PD-1 expression negatively correlates with the absolute CD4 cell count and we demonstrate that PD-1 expressing T cells are more responsive to PD-ligand mediated inhibition of T cell proliferation.
Conclusion: PD-1 is a unique marker for poor immunological recovery. We provide evidence that PD-1 mediated T cell suppression may have a role in impaired immune reconstitution in HIV patients.

P104

A protective role for B cells deficient in IL-4Ra in cutaneous leishmaniasis

Revaz-Breton M., Hoving J.C., Schwegmann A., Guler R., Arendse B., Brombacher F.
Institute of Infectious Disease & Molecular Medicine IIDMM, Health Science Faculty, UCT, Room S1.21; Werner Beith South Wing, 7925 Observatory, Cape Town, South Africa

Immunologic investigations on the murine model of infection with *Leishmania major* (*L. major*) have correlated the outcome of the disease with expansion of different subsets of CD4+ cells, designated Th1 and Th2. The resistance of C57BL/6 mice is linked with an IL-12 driven Th1 response. Whereas in BALB/c mice the susceptibility correlate with an IL-4 driven Th2 response. Previous studies in the lab have identified important roles played by IL-4 and IL-13 target cells in cutaneous leishmaniasis, in fact IL-4 receptor α (IL-4Ra) deficient BALB/c mice are able to control acute cutaneous leishmaniasis despite a sustained development of Th2 cells. This suggests that cells expressing IL-4Ra other than Th2 cells can contribute to the susceptibility. Different cell type specific for IL-4Ra deficient mice have been generated in order to further dissect the role of different IL-4 and IL-13 target cells in cutaneous leishmaniasis. More specifically the objective of this project is to understand the role of IL-4Ra responsive B cells in L. major infection. Several studies have shown that B cells are involved in susceptibility in *Leishmania* infection. B cells deficient mMT mice on a BALB/c background have shown to be resistant to the L. major LV39 strain. As mice deficient in IL-4Ra specifically in B cells are as resistant as mMT mice after L. major LV39 infection we can conclude that IL4Ra deficient B cells do not contribute to susceptibility after L. major infection. More importantly whereas mMT mice infected with L. major IL-81 parasite still develop lesion as high as BALB/c WT mice, preliminary results show that mice deficient in IL-4Ra specifically in B cells are resistant to this virulent parasite strain. Revealing that IL4Ra deficient B cells do not play a role in susceptibility but more importantly they may also be a factor of resistance in L. major infection. Our data infers that B cells responding to IL-4 and IL-13 are implied in susceptibility, whereas B cells which are unresponsive to IL-4/IL-13 (IL-4Ra deficient) are needed for an increased resistance to *Leishmania* infection. Further studies should allow uncovering the exact role of these cellular players in cutaneous leishmaniasis.

P105

Role of antigen presenting cells in CD8 T cell exhaustion during chronic viral infection

Richter K., Wolint P., Brocker Th., Oxenius A.
ETH Zurich, Institute for Microbiology and Immunology, Zurich, Switzerland

During persistent viral infection the immune system is challenged by the constant exposure to antigen potentially causing continuous activation of CD8 T cells which could induce immunopathology. Chronic lymphocytic choriomeningitis virus (LCMV) infection is marked by CD8 T cell exhaustion which is characterized by a strongly reduced cytokine secretion and loss of proliferative capacity of virus-specific CD8 T cells. The degree of CD8 T cell dysfunction and viral load correlate, but the role of antigen presentation by bone marrow derived versus parenchymal cells in T cell exhaustion is poorly understood. In the current study we address the hypothesis that antigen presentation by non-professional antigen presenting cells contributes to CD8 T cell exhaustion. Accordingly, exclusive antigen presentation by dendritic cells should lead to a lower degree of CD8 T cell exhaustion than presentation of viral antigens on different cell types including non-professional antigen presenting cells. To test this hypothesis we made use of β2-microglobulin knockout mice transgenically expressing MHC I under control of the CD11c promoter (designated KM14 mice) in which CD8 T cells can only recognize their antigen selectively on dendritic cells. Chronic LCMV infection leads to similar virus titers in KM14 and wild-type mice and in accordance with our hypothesis to a decreased CD8 T cell exhaustion in KM14 mice. Similar effects were observed for LCMV-specific transgenic CD8 T cells which were transferred into KM14 respective wild-type mice prior to chronic LCMV infection. Unexpectedly, after adoptive T cell transfer, KM14 mice succumb to infection. In ongoing work we aim to characterize if the increased CD8 T cell functionality in chronically LCMV infected KM14 mice is the cause of the observed pathology and to determine whether the pathology and/or increased CD8 T cell functionality can be decreased by provision of high amounts of antigen presented on different cell types.

P106

IL-10 produced by B cells orientates the TH2 immune response in BALB/c mice infected with *Leishmania major*

Ronet C., Hauyon-LaTorre Y., Revaz-Breton M., Mastelic B., Tacchini-Cottier F., Louis J., Launois P.
WHO-IRTC, Dpt of Biochemistry, Epalinges, Switzerland

In the murine model of infection with *L. major*, the role of B cells is debated but recent evidences indicate that B cells are required for susceptibility to infection in BALB/c. In this study, we analyzed the role of cytokines such as IL-10 produced by B cells in the susceptibility of infection with *L. major*. We first demonstrated that in vitro B cells purified of splenic cells from BALB/c mice produced IL-10 in response to stimulation with *L. major*. In vivo, early IL-10 mRNA expression is detected in B cells of draining lymph nodes from BALB/c but not of C57BL/6 mice. Both in vitro and in vivo, the IL-10 producing B cells in response to *L. major* stimulation express specifically the CD1d and CD5 molecules suggesting that these B cells are regulatory B cells. Indeed, in contrast to adoptive transfer of naïve wild type B cells prior to infection in B cell deficient BALB/c mice that restore susceptibility to infection with *L. major* of these otherwise resistant mice, adoptive transfer of IL-10-/- B cells mice did not. We demonstrated that IL-10 produced by B cells is able to down-regulate the IL-12 production by *L. major* stimulated dendritic cells. Altogether these results demonstrated that IL-10 produced by regulatory CD1d+ CD5+ B cells in response to *L. major* stimulation is critical for Th2 cell development in BALB/c mice by regulating the IL-12 production.

P107

The poorly neutralized lassa fever virus envelope elicits a rapid and potent neutralizing antibody response against glycan-deficient viral variants

Sommerstein R., Flatz L., Bergthaler A., Pinschewer D.D.
University of Geneva, Pathology and Immunology, 1, Geneva 4, Switzerland

Old World arenaviruses like Lassa fever virus (LFV) and lymphocytic choriomeningitis virus (LCMV) typically elicit vastly delayed and weak neutralizing antibody (nAb) responses (~60 days after infection). Here we tested the hypothesis that dense glycosylation of the above viral envelopes may inhibit the induction and/or the antiviral efficiency of the host's antibody response.
Experimental approach and results: We studied the antibody response of mice to LCMV and to a recombinant thereof expressing the LFV glycoprotein (rLCMV/LFV GP). Mutants of these viruses were also tested that lacked single or multiple N-linked glycosylation sites in the outer glycoprotein (GP) domain GP1. We observed, that with a gradually decreasing number of glycosylation sites in GP1, neutralizing Antibody (nAb) responses developed more rapidly and to higher titers. To our surprise, however, we found that also normally glycosylated virus elicited a comparably rapid and potent antibody response against its glycan-deficient variants. Similarly, "deglycosylated" LCMV variants were neutralized more efficiently by monoclonal antibody (mAb) than their normally glycosylated parent virus. This concept was further extended to Junin virus, the causative agent of Argentine hemorrhagic fever, suggesting that glycosylation may represent a general mechanism of arenavirus antibody evasion.
Conclusions: Envelope-specific antibodies against LCMV and LFV are rapidly induced, but N-linked glycans prevent them from neutralizing the respective wild type viruses. This viral immune evasion strategy delineates important limitations for antibody-based vaccination.

P108 CANCELLED

Efficient *listeria* clearing needs collaboration between TLR and integrin on antigen presenting cells

Togni M., Reinhold A., Schlüter D., Reinhold D., Schraven B.
Institute for Molecular and Clinical Immunology, Otto-von-Guericke-University, Magdeburg, Germany

Listeria monocytogenes is a facultative intracellular bacterium which may cause severe infections in immunocompromised patients, very young and old persons, and pregnant women. It is generally acquired by the ingestion of contaminated food. Although the way of infection is clear, the exact mechanisms leading to the efficient elimination of the pathogen are only partially understood. Here, we report that mice lacking the cytosolic adapter molecule Skap-hom are more sensitive to listeriosis. In particular, Skap-hom-deficient mice develop a reduced number of *Listeria*-specific CD8 T cells and cannot completely clear the pathogen. This seems to be related to the fact that skap-hom-deficient dendritic cells, the most potent antigen presenting cells (APC), show an altered proximal signalling following the encounter of the pathogen, at lesat in vitro. In fact, TLR signalling seems to be unaltered since stimulation of DC results in Ikb degradation in both wild type and Skap-hom deficient DCs. However, downstream of integrins, Pyk2 and Fak are not activated in the absence of Skap-hom. These data suggest that both, TLR and integrin signalling on the APC, are needed for an efficient activation of the adaptive immune system and for an efficient clearing of the pathogen.
The studies are supported by DFG grant Schr 533/5-3

P109

The murine cytomegalovirus (MCMV) specific CD4 T cell response controls viral replication by exerting direct antiviral mechanisms

Walton S.M., Torti N., Zimmermann A., Hengel H., Hill A.B. and Oxenius A.
ETH Zurich, Institute of Microbiology, Zurich, Switzerland

Cytomegaloviruses establish a latent and persistent infection. Previous studies showed that the innate as well as the adaptive immune response are crucial to control MCMV infection. Although NK cells, B cells and CD8 T cells are important cell subsets to control primary and latent infection, CD4 T cells are crucial for establishment of viral latency in salivary glands (SG). However, the exact mechanisms how CD4 T cells contribute to viral control in this particular organ are not well understood. Taking advantage of different mouse models we show that CD4 T cells do not exert antiviral functions via helper mechanisms: in the absence of CD8 T and B cells, viral clearance was comparable to wild type animals. However, a role of B cells in the control of viral spreading as previously proposed cannot be excluded as mice lacking CD4 T cells and concomitantly IgG antibodies show higher viral titers in late stages of MCMV infection than mice lacking CD4 T cells in the presence of normal MCMV-specific IgG titers. Hence, CD4 T cells control MCMV replication in the SG mainly by exertion of direct antiviral effector functions. Using different bone marrow chimeric mice, we show that IFNγ but not perforin produced by CD4 T cells plays a major role in the establishment of MCMV latency. Further, IFNγ seems to act directly on infected cells in the SG as deficiency of IFNγ receptor on radiation resistant cells, (hence cells that are most likely infected in the SG), leads to a decrease of viral control. Currently we examine the role of TNFα secreted by CD4 T cells in the control of MCMV replication and we address the question why IFNγ and TNFα producing MCMV-specific CD8 T cells, which are found abundantly in the SG of infected mice, are unable to control viral replication comparable to their CD4 counterparts.

P110

Cytomegalovirus and immune senescence: vaccination efficacy is impaired in CMV-infected elderly individuals

Wanke K., Von Braun A., Häberli L., Mukhopadhyay D., Schaffner T., Bosshart W., Stiasny K., Heinz F.X., Held L., Steffen R., Karrer U.
Division of Infectious Diseases, University Hospital of Zurich, Zurich, Switzerland

Immune senescence contributes to decreased resistance of elderly individuals against infectious diseases and impairs protective immunity after vaccination. Recent circumstantial evidence has linked infection with herpesviruses in general and Cytomegalovirus (CMV) in particular with premature immune senescence.
Aims: We tested the hypothesis whether and how CMV-infection affects protective immunity after vaccination in healthy elderly individuals.
Methods: We performed a prospective, controlled study in 155 healthy elderly volunteers subdivided in two groups of CMV-positive and CMV-negative individuals and used vaccination efficacy as a surrogate marker for immune senescence. All participants were vaccinated against tick-borne encephalitis virus (TBEV) at 0, 4 and 24 weeks and TBEV-specific antibody and T cell responses were monitored longitudinally by ELISA, neutralisation assays and IFNγ ELISpot. Cellular immune responses against common herpesviruses including CMV were quantified and correlated with vaccination efficacy.
Results: TBEV-specific antibody titres measured by ELISA and neutralisation assay were two to threefold lower in CMV-positive than in CMV-negative elderly individuals after two (10.9 vs. 29.4; $p < 0.01$) and three doses of TBEV-vaccine (42.3 vs. 82.4; $p < 0.05$). Similarly, vaccine induced TBEV-specific T cell responses were significantly lower in CMV-positive participants. TBEV-vaccination efficacy was negatively correlated with the strength of cellular immunity specific for common herpesviruses and for CMV.
Conclusions: Our results demonstrate that CMV-infection has a negative influence on protective immunity after TBEV-vaccination in healthy elderly individuals. This suggests that persistent CMV-infection may be a relevant factor for the development of immune senescence. Correlation analyses suggest an immunologic mechanism of interference possibly involving T cell homeostasis and memory inflation.

P111

Antibodies protect from infection by targeting intracellular bacteria to the lysosomal compartment

Weber S., Joller N., Müller A.J., Spörri R., Hilbi H., Oxenius A.
ETH Zurich, Institute of Microbiology, Zurich, Switzerland

Adaptive immune responses mediated by antibodies (Abs) and T cells protect the organism from secondary challenges with pathogens. The protective effect of Abs is generally attributed to either neutralization, complement activation, or Ab-dependent cell-mediated cytotoxicity (ADCC). In this study we demonstrate that Abs protect from infection with Legionella pneumophila (Lpn), the causative agent of Legionnaire's disease, which normally evades lysosomal degradation by actively establishing a replication permissive vacuole. This protective effect was not mediated by complement, but rather by Ab-dependent targeting of the bacteria for degradation. In vitro experiments showed that opsonized Lpn are preferentially located in LAMP-1+ vacuoles and that intracellular replication in macrophages was inhibited. Furthermore, we show that targeting of the bacteria to lysosomes is dependent on the Fc portion of the Abs and on Fc receptors, indicating a role for Fc receptor mediated signalling in this protective mechanism. Finally the presence of specific Abs also targets Lpn into lysosomal compartments in vivo.

P112

The effects of two parasite antigens (*Toxoplasma gondii* and *Toxocara canis*) on WEHI-164 fibrosarcoma growth in mouse model

Yousofi Darani H., Shirzad H., Mansoori F.
Shahrekord University of Medical sciences, Parasitology, Dr. Hossein Yousofi Darani: Dept. of Parasitology, 88157-17311, Shahrekord

Cancer is the main cause of death in developed countries. However in underdeveloped countries infections and parasitic disease are the main causes of death. There are raising scientific evidences indicating that parasitic infection induces antitumor activity against certain types of cancers. In this study the effect of *Toxoploma gondii* and *Toxocara canis* egg antigens in comparison with BCG with known anticancer distinctives on WEHI-164 fibosarcoma transplanted to Balb/c mice was investigated.
Methods: Groups of 6 male Balb/c mice injected with *Toxoplasma gonidii* antigen, BCG or *Toxocara canis* egg antigen as case groups and alum alone as control groups. All mice were then challenged with WEHI -164 fibrosarcoma cells. The mice were examined for growth of solid tumor and the tumor sizes were measured every two days up to 4 weeks.
Results: The mean area of tumor in *Toxoplasma gondii*, BCG or alum alone injected mice in 4 different days of measurement were 25 mm^2, 23 mm^2 and 186 mm^2 respectively. Also the mean of tumor area in *Toxocara canis* injected mice in 4 different days was 24 mm^2 compared to control group (alum treated) which was 155 mm^2.
Conclusion: The mechanism by which, parasite antigens interfere with tumor growth is not clearly understood, it is possible that immune responses provoked by parasite antigens nonspecifically affect the tumor growth.

P113

Drug-resistant influenza virus induced by oseltamivir in an immunocompromised patient

Asner S., Thomas Y., Burnand J., Ernard V., Kaiser L., Meylan P.
Centre Hospitalier Universitaire Vaudois CHUV, Infectious Diseases Service, Lausanne, Switzerland

The occurence of oseltamivir resistance is well described in the immunocompromised host during therapy. Recently, oseltamivir Influenza A (H1N1) resistant strains have circulated in several European countries in which oseltamivir exposure is minimal.
Objective: To describe an immunocompromised patient in whom Influenza A (H1N1) acquired the H274Y neuraminidase resistance mutation while on oseltamivir treatment.
Results: A 48-year-old man admitted for autologous HSC transplant was tested positive for influenza A after starting chemotherapy. Oseltamivir was immediately initiated. Six days later he experienced persisting fever with worsening cough; a computer tomography (CT) showed interstitial pneumonia. Bronchoalveolar lavage (BAL) was performed and tested again positive for Influenza A (H1N1). In the absence of other source of fever, the hypothesis of oseltamivir-resistant influenza A was raised and oseltamivir was substituted by inhaled zanamivir. The initial nasopharyngeal swab and the BAL were sent to the National Reference Center for Influenza (Geneva) for further characterization. In both samples an Influenza A (H1N1) was cultured. Phylogenetic analysis of the hemagglutinin gene confirmed the close relationship between the above strains. No significant difference could be observed between both strains and strains isolated in the same area (Western Switzerland). However, the BAL strain isolated after 7 days of oseltamivir treatment showed the H274Y mutation associated with a 400 fold increase in oseltamivir IC50. Shortly after starting zanamivir he became asymptomatic.
Conclusions: The selection of a H274Y mutation under drug pressure in an immunocompromised host is possibly favoured by an impaired immune response in the context of high viral replication rate. Whether similar events explain the rapid emergence of oseltamivir-resistant influenza A (H1N1) in Europe remains unknown. Indeed, the local influenza surveillance system showed no phylogenetically closely related strains. Thus, it is likely that the present H1N1 virus is able to circulate in the absence of significant drug pressure.

P114

Actinobaculum spp.: new microoganisms? Clinical observation of 19 cases

Béguelin C., Lienhard R., Jacquet A., Tritten M.L., Siegrist H.H., Genné D.
HNE La Chaux-de-Fonds, Internal Medicine, La Chaux-de-Fonds, Switzerland

Introduction: Actinobaculum spp. are new species that have so far been isolated from human blood, urine and pus. Their importance has probably been underestimated and the different species underdiagnosed until today, as the laboratory needs to search them actively. The aim of this study is to examine their clinical relevance.
Methods: This retrospective study takes into consideration all known cases of Actinobaculum spp. infections identified since 2004 in the canton of Neuchâtel (169000 inhabitants), Switzerland. Strains were cultivated and isolated in the bacteriology laboratory in its routine procedure. Identification usually included a API 32 A gallery (bioMerieux) and 16S RNA gene sequencing.
Results: Twenty positive samples could be found in 19 patients: (11M/8F) of all ages (16–91), 10 urine (50%), 6 blood (30%), 1 blood and urine (5%), and 3 pus (15%). 12/13 (92%) cases of urinary tract infection (UTI) had an underlying pathology of the genitourinary tract. When urine cultures were positive for Actinobaculum spp., leucocytes were found in all samples but nitrite tests were mostly negative [6/7(86%)]. All samples showed Gram positive rods. Onset of concordant treatments were delayed by an average of 2.7 days (range 0–13 days), due to the diminished sensitivity of Actinobaculum spp. to the commonly used antibiotics in UTI (ciprofloxacine and sulfamethoxazol/thrimetoprime) and to the length of microbiological diagnosis. Fifty percent of the cases were treated as outpatients and 18/19 (95%) had a favorable outcome.
Conclusion: To our knowledge, this is the largest series published to date. In case of leukocyturia with a negative nitrite test but presence of Gram positive rods, in patients with an underlying genitourinary tract pathology, Actinobaculum spp. should specifically be searched instead of considering clinically irrelevant colonization by Corynebacteria. This infection is probably much more common than previously thought.

P115

Assessment of all-cause pneumonia admissions before introduction of the pneumococcal conjugate vaccine in Switzerland

Betsch B.-Y., Mühlemann K.
Institute for Infectious Diseases, University Bern, Department of Infectious Diseases, Inselspital, Inselspital Bern, PKT IIB, Bern, Switzerland

Objective: To describe the baseline epidemiology of hospitalized pneumonia before the introduction of the 7-valent pneumococcal conjugate vaccine in Switzerland in 2006.
Methods: National hospitalization data were obtained from the Federal Institute of Statistics for the years 1998–2006 including the primary diagnosis (first-listed; International Classification of Diseases), up to 7 additional diagnoses, and other parameters characterising hospitalisation. Community acquired pneumonia (CAP) was defined by a primary diagnosis of pneumonia or meningitis/septicaemia plus a code for pneumonia. Pneumococcal CAP (SpnCAP) was defined as CAP with a pneumococcal disease code. Hospitalization rates for CAP and SpnCAP were calculated by segmented regression analysis.
Results: There were 122'572 hospitalizations for CAP (annual average 15'322). SpnCAP was coded in 5.1% of CAP. CAP hospitalization rates showed a rising trend between 1998 and 2001 probably due to a reporting bias. Thereafter, annual CAP (SpnCAP) rates per 105 populations were stable at an average of 425 (21). Rates varied by age group with highest rates among the <2 years olds 362 (14) and the >80 years olds 1525 (64). Males predominated (57%) especially in the <2 years olds (58%), and the elderly (60%). Ethnicity was Swiss in 84% of CAP cases, but this proportion was lower for <2 years olds (73%) and increased with age to reach 90% in the elderly. Average hospital stay was 13 days, but stay was shortest for the younger age groups and increased with age from 6.1 days in the <2 years olds to 17 days in the >80 years olds. Case fatality rate was 7.4% overall with most (88%) fatal cases occurring in the elderly. Admission to intensive care treatment was needed in 6.2% of CAP.
Conclusion: Data for the pre-vaccine years 2002 to 2005 serve as baseline for evaluating the impact of conjugated pneumococcal vaccines.

P116

Transmission rate of enterobacteriaceae producing extended-spectrum beta-lactamase (esbl) to hospital contacts and household members in a Swiss university hospital

Betsch B.-Y., Droz S., Mühlemann K.
Institute for Infectious Diseases, University Bern, Department of Infectious Diseases, Inselspital Bern, PKT IIB, Bern, Switzerland

Objective: We studied the transmission rate of gram-negative bacteria with extended-spectrum beta-lactamase (ESBL) production from index patients with ESBL carriage to hospital room mates and household members.
Methods: Patients with ESBL carriage newly detected during diagnostic work-up were recruited prospectively during the time period May 7 to December 13, 2008. Hospital contacts were defined as room-mates for 48 hours. Screening was performed weekly for the duration of contact and continued for 2 weeks after separation from the index patient until 2 negative results were obtained. Screening included always a fecal sample; and in addition from patients a respiratory tract sample in case of intubation/tracheostoma, swabs from skin lesions and body fluids if drained by a catheter. Fecal samples were collected from household contacts in a 3-monthly interval until both index and contacts screened negative. Stool samples were analyzed with 3 different ESBL selective culture media: ChromID ESBL agar (Biomérieux) and ESBL agar (AES), a bi-plate with 2 selective media (MacConkey agar plus Ceftazidim and Drigalski agar plus Cefotaxim).
Results: 37 index patients, 24 (65%) inpatients and 13 (35%) outpatients were analyzed. The ESBL-species detected was E. coli, K.oxycota and K. pneumoniae in 58%, 29% and 13% for inpatients and 77%, 15% and 8% for outpatients. Fecal carriage was detected in 75% of inpatients and in 54% of outpatients. ESBL carriage was detected in 5 of 16 households (7 of 27 members). 3 of these 5 households were of south-asian ethnicity. Fecal ESBL carriage of index patients was 80% in 5 households with and 55% in the 11 households without ESBL transmission.
Conclusions: The rate of fecal ESBL carriage tends to be higher in hospitalized than outpatients (75% vs. 54%). Inhospital patient-to-patient transmission rates may be lower than transmission rates within households and may correlate with fecal carriage in the index patient.

P117

Usefulness of a routine monitoring of cytomegalovirus viremia after discontinuation of antiviral prophylaxis in kidney transplantation

Boillat N., Pascual M., Venetz J.-P., Meylan P.-R., Manuel O.
CHUV, Infectious Diseases and Transplantation Center, BH08 652 CHUV, Lausanne, Switzerland

Late-onset cytomegalovirus (CMV) disease after discontinuing antiviral prophylaxis remains a cause for significant morbidity in solid organ transplant (Tx) recipients. The aim of this study was to assess the usefulness of a routine preemptive post-prophylaxis (PP) strategy.
Method: Between November 2003 and November 2007, kidney Tx recipients with any CMV seropositive donor/recipient (D/R) combination were included. All patients received a three-month prophylaxis with valganciclovir, followed by a 3 to 4 months preemptive PP strategy, involving CMV viral load testing by quantitative PCR every 15 days. Antiviral therapy was initiated using a positive cut-off of >10'000 copies/106 (= 1e06) white blood cells (WBC). Patients were followed up to one year post transplant.
Results: A total of 89 consecutive patients were included. CMV serostatus was: D+/R+: n = 41, D-/R+: n = 18, D+/R-: n = 30. The mean age was 48.4 years. Overall, 25% received induction therapy with thymoglobulin and 75% received basiliximab. A median of 6 PP PCR per patient were performed. At 6 months post-transplant (3 months PP), CMV infection (viremia disease) occurred in 31/89 (35%) patients: 18/59 (31%) in the R+ group and 13/30 (43%) in the D+/R- group (p = 0.25). Median (range) peak viral load was 277 (19–9750) and 14'000 (33–932'000) copies/106 (= 1e06) WBC in R+ and D+/R- patients (p = 0.004), respectively. Incidence of CMV disease was 0/59 in the R+ group and 7/30 (23%) in the D+/R- group (p <0.001). In the R+ group, all positive PCR were below the cut-off and patients were not treated. In the D+/R- group, among the 13 patients with CMV infection, 5/13 (38%) developed CMV disease simultaneously to the first positive viral load, 2/13 (15%) patients received a pre-emptive treatment and did not develop CMV disease, 2/13 (15%) were not treated and eventually developed CMV disease and 4/13 (31%) never developed CMV disease. Two additional D+/R- patients developed CMV disease between 6 and 12 months post-Tx.
Conclusion: A preemptive PP strategy was not useful in intermediate-risk seropositive patients because of the absence of significant viremia or CMV disease. In the high-risk D+/R- group, such a strategy appears to be useful as it can prevent cases of late-onset CMV disease. These results have implications to determine the cost-effectiveness of CMV surveillance strategies.

P118

Multiple colonisation with *S. pneumoniae* in the nasopharynx

Brugger S.D., Hathaway L.J., Mühlemann K.
University of Bern, Institute for Infectious Diseases, Bern, Switzerland

There is a lack of techniques that detect multiple colonisation by S. pneumoniae directly in nasopharyngeal samples.
Methods: 287 nasopharyngeal swabs collected during the pre-vaccine era within a nationwide surveillance program were analyzed by a novel technique for the detection of co-colonisation based on PCR amplification of a non-coding region adjacent to the pneumolysin gene (plyNCR) and restriction fragment length polymorphism (RFLP) analysis. The number of strains and their relative abundance in co-colonised samples was determined by terminal RFLP (T-RFLP).
Results: Pneumococcal carriage rate was 51.6% by PCR as compared to 40.0% by culture. Co-colonisation was present in 9.5% (10/105) of samples, most (9/10) of which contained 2 strains in a ratio between 1:1 and 17:1. Five of the ten co-colonised samples showed a combination of vaccine types only (n = 2) or combinations of non-vaccine types only (n = 3). Carriers of multiple pneumococcal strains had more often received recent antibiotic treatment than those colonised with a single strain (33% versus 9%, p = 0.025).
Conclusions: This new technique allows for the rapid and economical study of pneumococcal co-colonisation in nasopharyngeal swabs. It will be valuable for the surveillance of S. pneumoniae epidemiology under vaccine selection pressure.

P119

Clinical guidelines improve initial management of community – acquired meningitis (CAM)

Chapuis-Taillard C., Manuel O., Meylan P., Bille J., Calandra T., Marchetti O.
CHUV, Lausanne, Switzerland

Background: Prompt work-up and rapid initiation of appropriate therapy are crucial in CAM. The aim of the study was to assess if clinical guidelines improve the initial management of CAM.
Methods: 52 consecutive adults admitted during 2005–7 at CHUV after implementation of CAM guidelines (decision algorithm for indication and timing of lumbar puncture (LP), blood cultures, head CT-scan, antibacterial (AB) and corticosteroid therapy) were compared with 50 CAM cases during 2002–5 (no guidelines). Initial management was evaluated: *– composite guidelines application score (0 to 4): – LP before AB in absence of contraindications (1), – blood cultures before AB (1), – appropriate timing of AB therapy after/before LP (1), – corticosteroids before/simultaneously with AB therapy (1), – indicated head CT-scan before LP (altered consciousness, focal signs, extrameningeal focus).*
Results: Demographics and clinical characteristics (age, sex, duration of symptoms, headache, fever, neck stiffness, Glasgow score) were similar in the guidelines vs. no guidelines groups. The proportion of bacterial/aseptic CAM was 23%/77% and 46%/54%, respectively. Overall, initial management was improved in the guidelines group (score ≥2 in 56% vs 34%, p = 0.04; ≥3 in 36% vs 10%, p = 0.004.) In the sub-group of bacterial CAM: initial management was also improved in the guidelines group (management score ≥2 in 92% vs 43.5%, p = 0.01; ≥3 in 58% vs 17%, p = 0.02, median time to LP 2h vs 5h, p = 0.05, median time to adequate antibiotherapy 1h vs 4 h, p = 0.09, corticosteroids administration 83% vs 43.5%, p = 0.03, and indicated head CT scan before LP 89% vs 55%, p = 0.04). In bacterial CAM no difference was observed for ICU stay (median 3d, 2 to 5 vs 2, 1 to 9) or in-hospital survival (90 vs 96%), respectively.
Conclusions: Clinical guidelines significantly improve the initial sequence of investigations and antiinfective/antiinflammatory therapy in community-acquired meningitis. Their impact on morbidity/mortality needs to be investigated in a larger population.

P120

A pain in the neck – probiotics for ulcerative colitis

Conen A., Trampuz A., Zimmerer S., Frei R., Battegay M., Elzi L.
Infectious Diseases and Hospital Epidemiology, Internal Medicine, University Hospital Basel, Basel, Switzerland

Case report: A 38-year old woman presented in May 2008 with a 4-week history of progressive neck pain irradiating to the head. The patient had been suffering from severe ulcerative colitis since 2002 with increasing activity over the last 5 months. Prior treatments included high-dose steroids (recently prednisone 40 mg daily), TNFα blockers, azathioprin and cyclosporine. Because of persistent bloody diarrhoea and abdominal cramps a dairy product containing probiotics (Aktifit™) was added in March 2008 in order to boost immunity. On admission the patient had fever, impairment of cervical spine mobility with no neck stiffness or neurological deficits. WBC was 10x109/L and C-reactive protein 150 mg/L. Blood cultures remained sterile. MRI showed a cervical epidural and a retropharyngeal abscess. Echocardiography revealed no signs of endocarditis. Both abscesses were drained and culture yielded Lactobacillus rhamnosus (resistant to cephalosporins class I–IV and carbapenems) and Candida kefyr. Given the microbiological results and the history of probiotic consumption we also cultivated Aktifit™, yielding L. rhamnosus with identical genetic sequencing pattern and resistance testing. Treatment with imipenem was started empirically, followed by clindamycin and fluconazole according to susceptibility tests. Antibiotic and antifungal therapy was continued for 3 and 6 months, respectively, with favourable recovery.
Discussion: Spinal epidural abscess is a life-threatening infection, usually occurring secondary to hematogenous dissemination from distant foci. We demonstrated the causal association between L. rhamnosus from the abscesses and Aktifit™ by genetic analysis. The source of C. kefyr is presumably related to the consumption of other dairy products. The use of probiotics in medical practice is rapidly increasing. However, recommendations rely on few clinical trials with methodological limitations and no data exist in the setting of immunosuppression.
Conclusions: The use of probiotics in medical practice deserves closer attention and careful individual evaluation, as probiotics may cause life-threating infections especially in immunocompromised individuals.

P121

Outcome of vascular graft infection

Evison J.-M., Bianchi A., Widmer M.-K., Mühlemann K.
University Hospital Bern, University Clinic for Infectious Diseases, Poliklinik für Infektiologie und Reisemedizin PKT, Bern, Switzerland

Infections of vascular grafts are a serious complication of graft surgery. Data about outcome and factors promoting healing are scarce.
Methods: A retrospective analysis was conducted at a tertiary care centre for the years 1998 to 2005. Included were all patients diagnosed with a surgical site infection in association with a vascular graft according to the definitions of the Centers for Disease Control and Prevention (CDC). Data were abstracted from clinical charts. Follow-up was a minimum of 24 months after diagnosis. Healing was defined as absence of clinical, radiological and intra-operative signs of infection and sterile cultures from intra-operative specimens (if applicable).
Results: A total of 93 patients were included, 26 (28%) had a superficial infection affecting the skin and subcutaneous tissue

overlying the graft, and 67 (72%) had a deep or organ space infection. Average age was 66.9 years (SD ± 11.8), and males predominated (76%). Infected grafts included abdominal aorta and aorto-bi-iliacal bypasses (19%, n = 18), aorto-iliacal-peripheral bypasses (29%, n = 27), femoro-peripheral bypasses (51%, n = 47) and wallstent (1%, n = 1). Patients were followed for an average of 33.6 months (SD ± 22.5). Overall healing rate was 70.9%. Factors favouring healing were superficial infection (healing rate 92.3% versus 62.6% for deep or organ space infection), longer duration of symptoms (>3 weeks) before diagnosis, no necessity for emergency surgery, absence of graft thrombosis or rupture, and treatment with statins at time of graft implantation or infection. Superficial infections had high healing rates when treated with debridement and relative short courses of antimicrobial treatment (median 29 days, range 0 to 120 days). Infections with a Samson classifications score ≥3 profited from complete graft removal or replacement.
Conclusions: Superficial vascular graft infections have an excellent prognosis. Characteristics of presentation of infection and graft complications seem to be more important for the outcome of vascular graft infection than length of antibiotic treatment.

P122

Teaching intervention improves compliance with hand disinfection among nurses but fails among physicians

Flammer Y., Giger H., Ruef C.
Div. of Infectious Diseases and Hosp. Epidemiology, HAL 14C, University Hospital of Zurich, Zurich, Switzerland

To investigate the impact of teaching interventions on the compliance with hand disinfection (HD-C) of physicians and nurses.
Methods: The study was carried out on two intervention (IW) and one control ward (CW). During weeks one and six, hand disinfection (HD) performance was directly observed on IW and CW. During weeks two to five, nurses and physicians attended teaching sessions on IW (nurses: 3 to 5 sessions [20 minutes each]/week, physicians: 1–2 sessions [10 minutes, general discussion of nosocomial infections, importance of HD/week]), whereas no teaching was offered to the CW. Teaching of nurses included visualising the efficacy of HD by ultraviolet light, education of 6 indications of HD, analysing daily activities for indications of HD, discussion of cases of nosocomial infections, and reporting results of hand cultures of health care workers.
Results: The overall HD-C of nurses was significantly improved (85.3%) despite an already relatively high baseline rate (75.9%). HD-C on CW remained unchanged. After intervention nurses showed a significant improvement in HD after contact with body secretions (baseline 69.4%, after intervention 86.9%). Overall compliance as well as compliance with individual indications of HD remained unchanged among physicians. There was no significant difference between nurses and physicians regarding overall HD-C at baseline (physicians 68.8%), but overall compliance was significantly higher among nurses than physicians (63.9%) after intervention.
Conclusion: Overall HD-C of nurses was improved by teaching. The teaching approach used in this study was successful in nurses but failed among physicians.

P123

Management of prosthetic joint infection: evaluation of procedures and outcome at a peripheral orthopaedic centre over a 4-year period

Gämperli A., Kaiser Ph., Schüler M., Krause M.
Kantonsspital Münsterlingen, Internal medicine / Infectiology, Scherzingen Switzerland

Prosthetic joint infections are a devastating complication requiring close collaboration of orthopaedic surgeons and infectious diseases specialists. Although standardized algorithms of diagnosis and treatment have been proposed, most surgeons maintain their traditional approach and management to combat these infections. We reviewed retrospectively the diagnostic and therapeutic strategies of prosthetic joint infections at our institution and compared the management with published guidelines. In the 4 years period 2003–2007, 1581 prosthetic joint replacements (1016 hip, 506 knee, 59 shoulder) were performed. Totally, 30 prosthetic joint infections were registered (1.9%), of which 21 occurred in hip (2.1%), 7 in knee (1.4%) and 2 in 59 shoulder implants (3.4%). Eleven episodes occurred as early (<3 months after surgery), 7 as delayed (3–12 months) and 12 as late infections (>12 months). Median patient age was 73 (47-94) years. The most common pathogen was Staphylococcus aureus (19 of 30, 63%), followed by coagulase-negative staphylococci. All patients underwent revision surgery. In 22 patients the implant was not removed, in 6 patients a two-stage exchange with temporary implantation of a spacer was chosen. One-stage exchange of the prosthesis was performed in only 2 cases. Antibiotic treatment was given upon resistance testing over a mean duration of approximately 3.6 months (mean 3.4 months for knee, 3.5 months for hip and shoulder). Three patients died before completion of the treatment due to cardiopulmonal co-morbidity; one patient needed further treatment for relapsing infection after 49 months and in two patients long-term antibiotic suppression therapy was chosen. In the remaining 26 patients, the infection appeared well controlled as long as follow-up was possible (median 25 months; 10–59). According to published treatment recommendations (Infection 31:99 and NEJM 351:1645) the surgical procedure chosen was adequate in 14 cases (47%), the prosthesis would have been retained in 5 (17%) and would have been removed in 11 patients (36%). We conclude that at our hospital, the outcome of prosthetic joint infections is favourable over all. Adherence to an algorithm defining a rational surgical and antibiotic treatment strategy may improve the multidisciplinary management and facilitate comparison with other institutions.

P124

High cefepime plasma concentrations and toxic encephalopathy in febrile neutropenic patients with mild impairment of renal function

Lamoth F., Buclin T., Pascual A., Vora S., Bolay S., Decosterd L.A., Calandra T., Marchetti O.
Centre Hospitalier Universitaire Vaudois, Infectious Diseases Service, Lausanne, Switzerland

High-dose cefepime therapy (6 g/d per 100 ml/min GFR) is recommended in febrile neutropenia. Cefepime-related encephalopathy has been associated with severe renal dysfunction. A reassessment of cefepime safety is ongoing on request of regulatory authorities due to the increased risk of mortality reported in a recent meta-analysis. This study aimed at investigating the association between cefepime plasma concentrations and safety in febrile neutropenic patients.
Methods: Cefepime trough concentrations (HPLC) were analyzed in 30 adult febrile neutropenic patients. The association between cefepime overdosing and toxic encephalopathy was assessed on the basis of high trough levels (>15 mg/L) and the presence of consistent neurological signs (NCI criteria).
Results: Median cefepime concentrations were 8.7 mg/L (range 2.1–38) at a median of 4 days (2–15) after start of therapy. Neurological signs (confusion/hallucinations/myoclonia) were attributed to high cefepime troughs in 6/30 (20%) patients (median GFR 45 ml/min, range 42–65) receiving a median dose of 13.2 g/d per 100ml/min GFR (9.2–14.3). Cefepime discontinuation resulted in complete neurological recovery in 5 patients and transient improvement in one, who died due to a progressive encephalopathy. The cefepime dose was preventively reduced in 5/30 (17%) patients due to high concentrations.
A regression logistic model confirmed high cefepime concentrations as an independent predictor of neurotoxicity: 50%-probability threshold at >22 mg/L (p = 0.01).
Conclusion: High cefepime plasma concentrations are associated with toxic encephalopathy in febrile neutropenic patients with mild renal dysfunction. Careful adherence to dosing recommendations is crucial. Monitoring plasma concentrations may contribute to prevent toxicity of high-dose therapy for this life-threatening condition.

P125

Ganciclovir exposure under a 450 mg daily dosage of valganciclovir for cytomegalovirus prevention in kidney transplant recipients: a prospective study

Manuel O., Pascual M., Perrottet N., Lamoth F., Venetz J.P., Decosterd L., Buclin T., Meylan P.
CHUV, Infectious Diseases and Transplantation Center, BH08 652 CHUV, Lausanne, Switzerland

It is suggested that a low dose of valganciclovir can be equally effective than a standard dose for cytomegalovirus (CMV) prophylaxis after kidney transplantation. The aim of our study was to determine the ganciclovir exposure observed under a routine daily dosage of 450 mg valganciclovir in kidney transplant recipients with a wide range of renal function.
Methods: In this prospective study, kidney transplant recipients with a GFR MDRD above 25 mL/min at risk for CMV (donor or recipient seropositive for CMV) received a dose of valganciclovir (450 mg daily) prophylaxis for 3 months. Ganciclovir levels at trough (Ctrough) and at peak (C3h) were measured monthly. Ganciclovir exposure (AUC0-24) was estimated using Bayesian non-linear mixed-effect modelling (NONMEM) and compared between 3 groups of patients according to their kidney function: GFRMDRD 26–39 mL/min (Group 1), GFRMDRD 40–59 mL/min (Group 2) and GFRMDRD 60–90 mL/min (Group 3). CMV DNAemia was assessed during and after prophylaxis using PCR.
Results: Thirty-six patients received 450 mg daily of valganciclovir for 3 months. Median ganciclovir C3h was 3.9 mg/L (range: 1.3–7.1) and Ctrough was 0.4 mg/L (range 0.1–2.7). Median (range) AUC0-24 of ganciclovir was 59.3 mg.h/L (39.0–85.3) in Group 1 patients, 35.8 mg.h/L (24.9–55.8) in Group 2 patients and 29.6 mg.h/L (22.0–43.2) in Group 3 patients (p <0.001). Anemia was more common in Group 1

patients compared to patients on the other groups (p = 0.01). No differences in other adverse events according to ganciclovir exposure were observed. CMV DNAemia was not detected during prophylaxis. After discontinuing prophylaxis, CMV DNAemia was seen in 8/34 patients (23.5%) and 4/36 patients (11%) developed CMV disease.
Conclusion: A routine dosage of valganciclovir achieved plasma levels of ganciclovir in patients with GFR>60 mL/min similar to those previously reported using oral ganciclovir. A daily dose of 450 mg valganciclovir appears to be acceptable for CMV prophylaxis in most kidney transplant recipients.

P126

Exclusive *Staphylococcus aureus* throat carriage: who is at risk?

Mertz D., Frei R., Periat N., Zimmerli M., Battegay M., Flückiger U., Widmer A.F.
University Hospital Basel, Division of Infectious Diseases, Basel, Switzerland

Approximately 25% of Staphylococcus aureus (S. aureus) carriers are exclusive throat carriers. We aimed to identify the population at risk for exclusive throat carriage to improve sensitivity to detect carriers.
Methods: Nasal and throat screening for S.aureus in four different groups. Three groups of individuals in the community (n = 2632) with different estimated levels of exposure to the health care system (HCS) were screened: 1500 healthy blood donors, 634 participants of a trade fair and 498 patients from the school of dental medicine. In-patients and health care workers formed the fourth group (n = 832) that were considered as the group with the highest estimated exposure to HCS. As primary outcome, we analyzed risk factors for exclusive throat carriage by comparison of exclusive throat versus all nasal carriers.
Results: Of 3464 individuals screened, 428(12.4%) were exclusive throat carriers and 1260 (36.4%) were colonized in the nares only or in the nares and the throat. The most important independent risk factor for exclusive throat carriage was age ≤30 years (OR 1.66, p <0.001). Exposure to HCS was a significant protective factor for exclusive throat carriage (OR 0.67, p = 0.001). Healthy blood donors were almost twice as likely to be exclusive throat carriers than hospitalized patients (30.2% and 18.4% of all carriers, p <0.001).
Conclusions: Absence of exposure to the HCS and young age were found to predict exclusive throat carriage, a population at high risk for community-onset methicillin-resistant S. aureus. Screening for S.aureus should include swabs from both the anterior nares and the throat to improve the likelihood to detect carriers.

P127

Septi*Fast*® for molecular diagnosis of sepsis in patients presenting to the emergency room

Schaub N., Steinhuber A., Boldanova T., Noveanu M., Stelzig C., Hermann H., Frei R., Siegemund M., Bingisser R., Trampuz A., Mueller C.
University Hospital, Basel, Switzerland.

Background: Blood cultures (BC) identify the etiology of sepsis in only a minority of patients. We assessed the utility of the multiplex real-time PCR Septi*Fast*® (SF) (Roche Molecular Systems) to detect microbial DNA of the 25 most important bacterial and fungal pathogens of sepsis within 6 h.
Methods: In this prospective ongoing study, we included unselected adult patients presenting to the emergency room with suspected sepsis, defined as core temperature >38.3 °C or <36.0 °C and ≥1 additional SIRS criterion. BC and SF were simultaneously drawn at presentation and 0.5–2 h thereafter. The final diagnosis of sepsis or non-infectious SIRS was adjudicated by 2 independent infectious diseases specialists not aware of the SF result.
Results: To date (06/07-12/08), 59 patients were included; 49 (83%) had sepsis and 10 (17%) had non-infectious SIRS. Among patients with sepsis (median age, 65 years; 45% males), the causative organism was identified in 12 patients (25%) with BC (7 *E. coli*, 3 *K. pneumoniae*, 1 *S. aureus*, 1 *Streptococcus pyogenes*). Median time to positivity (TTP) of BC was 24 h (range 6–45 h). SF revealed the causative organism in 12 patients with sepsis, of which 10 matched the BC result. 2 additional samples were positive in SF only (*E. coli*–cholangitis, S. *aureus* – primary *S. aureus* sepsis) and 2 samples were positive in BC only (*E. coli*-urospesis, *Streptococcus pyogenes*–erysipelas). In the 2 patients with false-positive BC (both with *coagulase-negative staphylococci),* SF remained negative.
Conclusions: SF and BC detected the causative organism of sepsis in 25%. Both assays missed 2 pathogens (BC: *S. aureus* and *E. coli*, SF: *S. pyogenes* and *E. coli).* In the 2 patients with contamination of BC, SF was negative, resulting in a better specificity (93 vs. 88%). SF warrants further evaluation for the same-day diagnosis of sepsis in the emergency department.

P128

Molecular epidemiology of methicillin-resistant *S. aureus* in roommates of newly identified carriers

Senn L., Bellini C., Zanetti G., Blanc D.S.
CHUV, Service de Médecine Préventive Hospitalière, Lausanne, Switzerland

Few epidemiological data are available about transmission of methicillin-resistant S. aureus (MRSA) from hospitalized colonized or infected carriers to their roommates. We conducted a 3-year prospective survey to evaluate MRSA cross-transmission among roommates.
Methods: Setting: 850-bed, tertiary care hospital with 11% of MRSA among clinical S. aureus isolates, and a search and destroy policy. Two thirds of MRSA belong to 4 predominant clones. We analyzed the results of MRSA screening swabs (nose, throat, groin) of roommates of newly identified MRSA patients. Roommates were patients hospitalized in the same room of a MRSA carrier for >24 h, from 48 h before the positive sample to the first day of contact precautions. Double locus sequence typing (DLST), a new typing strategy for MRSA that uses the repeat sequences of clfB and spa genes, was performed on isolates recovered from index cases and positive roommate contacts.
Results: From Jan. 2005 to Dec. 2007, 305 investigations including 942 roommates (median 3 roommates per investigation, range 1 to 15) were conducted. 49/305 (16%) investigations yielded 58/942 (6%) positive contacts. 45/58 (78%) positive contacts harbored a DLST-identical strain to the index case. Thus, possible cross-transmission occurred in 45/942 (4.8%) contacts, whereas a cross-transmission was excluded for the other positive roommates. Of note, 53/58 (91%) isolates belonged to predominant clones.
Discussion: In our setting, cross-transmission of MRSA occurred in only 4.8% of roommates of a MRSA carrier. This rate could be even lower, as a cross-transmission cannot be proven among patients harboring MRSA that belong to predominant clones. In spite of the predominance of 4 clones, molecular epidemiology allowed to exclude cross-transmission in 22% of cases. The source of MRSA remains unknown in these later cases.
In conclusion, MRSA screening of roommates contacts in endemic situations should be interpreted with caution in terms of cross-transmission.

P129

Postoperative serum Pro-Calcitonin and C-reactive protein levels in patients with orthopaedic infections

Uçkay I., Ferry T., Harbarth S., Garzoni C., Lew D.
Geneva University Hospitals, Service of Infectious Diseases, Geneva, Switzerland

The value of postoperative Pro-Calcitonin (PCT) levels in the follow-up of infected patients is unknown. The objective of this study was to compare the postoperative ultra-sensitive serum PCT and C-reactive protein (CRP) levels in infected orthopaedic patients and assess the utility of PCT in predicting the need for surgical re-intervention.
Methods: Retrospective study including adult orthopaedic patients at the Geneva University Hospital.
Results: A total of 165 paired PCT and CRP samples were retrieved in 60 patients (median age, 58 years, 17 females). Twenty-four patients required surgical re-intervention. Postoperative PCT values were elevated only in 15 patients. Median PCT levels exceeded normal only on the first postoperative day, despite a clinically active infection. PCT values did not differ between patient groups with one or more surgical interventions (Wilcoxon-ranksum-test, p = 0.33). CRP was elevated in 54 patients (90%), and normalized by the tenth day. Although paired samples, both markers correlated poorly with each other (Kendall-tau-test 0.47).
Conclusion: PCT has no predictive value for surgical re-interventions in patients with localized orthopaedic infections.

P130

Use of serum antistreptolysin O-titers in the microbial diagnosis of orthopaedic infections

Uçkay I., Ferry T., Ambrosioni J., Andrey D., Bernard L., Lew D.
Geneva University Hospitals, Service of Infectious Diseases, Geneva, Switzerland

The utility of serologic tests in the microbial diagnosis of orthopaedic infections is unknown. Antistreptolysin-O titer determination is inexpensive and accurate in the diagnosis of beta-hemolytic group A, C and G streptococci.
Methods: Retrospective analysis in the Geneva Universitxy Hospitals including adult patients with orthopaedic-related infections and ASO titer samples during hospitalization for that infection.
Results: Data was retrospectively retrieved for 21 patients (7 females, 14 males; median age, 64 years). Five patients had elevated ASO titers (10 specimens; median titer 600 U/ml, range 300–800 U/ml). The pathogens documented in the patients with elevated ASO titers were

S. pyogenes (beta-hemolytic streptococci of Lancefield group A; n = 3), beta-hemolytic streptococci of group G (n = 1) and beta-hemolytic streptococci of group C (n = 1). All five patients with elevated ASO titers were treated with intravenous penicillin or amoxicillin. All were cured with no recurrence during a follow-up period of at least three months.
Conclusion: In patients with negative culture results and positive titers antibiotics might be reduced to the narrowest spectrum, penicillin.

P131

Low utility of methicillin-resistant *Staphylococcus aureus* screening to predeict the Staphylococcal species in orthopaedic implant infections

Uçkay I., Ferry T., Lübbeke A., Bernard L., Harbarth S.
Geneva University Hospitals, Service of Infectious Diseases, Geneva, Switzerland

Staphylococcal implant infections are frequent. In case of concomitant skin colonisation of methicillin-resistant Staphylococcus aureus (MRSA), vancomycin is empirically prescribed while awaiting the microbiological results.
Methods: Retrospective study of staphylococcal orthopaedic implant infections. MRSA screening (PCR and/or culture) from nares and groin were performed at maximum two weeks before microbiological diagnosis by intraoperative specimens.
Results: A total of 102 staphylococcal infections were retrieved (38 due to MRSA, 33 due to methicillin-sensitive S. aureus (MSSA), and 31 due to coagulase-negative staphylococci (CoNS). In 8/33 (24%) cases of MSSA and 4/31cases (13%) of CoNS infections, the patients were screened MRSA positive on their skins. But 42% (16/38) of MRSA infections had negative MRSA screening results. The sensitivity and specificity of MRSA skin colonisation towards MRSA implant infections were 58% and 81%.
Conclusion: MRSA skin colonisation cannot predict the nature of underlying staphylococcal orthopaedic implant infection.

P132

Features of orthopaedic implant infections due to different staphylococci

Uçkay I., Teterycz D., Ferry T., Bernard L., Lew D.
Geneva University Hospitals, Service of Infectious Diseases, Geneva, Switzerland

Background: Comparison of clinical impacts of different staphylococci in orthopaedic implant infections is rare.
Objectives: To assess features of orthopaedic implant infections due to methicillin-resistant S. aureus (MRSA), methicillin-sensitive S. aureus (MSSA), and coagulase-negative staphylococci (CoNS).
Methods: Retrospective study in the Geneva University Hospitals.
Results: There were 43 episodes due to MRSA, 49 due to MSSA, and 55 due to CoNS. Total cure was achieved in 60% (26/43), in 71% (35/49), and in 85% (47/55), respectively. In multivariate analysis, CoNS was significantly associated with microbiological and functional cure (OR 3.8, 1.5–10.1), while S. aureus (MRSA & MSSA) was negatively associated (OR 0.3, 0.1–0.8); without separate differences between MRSA and MSSA. CoNS infections involved rather big implants such as arthroplasties or intramedullar nails and were more frequently followed by a new implant than S. aureus (CHI;2-tests, p <0.01). Methicillin-resistance per se, immunosuppression, sex, age, duration of antibiotic therapy, implant removal, the proportion of one-stage revision, rifampin use and the number of surgical interventions did not influence cure. Clinically, S. aureus was more virulent than CoNS with a shorter incubation time, more bacteraemia, and more surgical interventions (CHI;2, Wilcoxon-ranksum-test, p <;0.03).
Conclusions: In orthopaedic implant infections, S. aureus is more virulent than CoNS and is independently associated with a worse microbiological and functional cure. There are no significant differences between MRSA and MSSA.

P133

Low incidence of haematogenous seeding to total hip and knee arthroplasties in patients with remote infections

Uçkay I., Lübbeke A., Emonet S., Tovmirzaeva L., Ferry T.
Geneva University Hospitals, Service of Infectious Diseases, Geneva, Switzerland

The exposure of arthroplasty patients to remote infections is unknown.
Objective: To investigate the incidence of secondary arthroplaty infections. To estimate the burden of remote infections without seeding to arthroplasties.
Methods: Prospective cohort study of elective hip and knee arthroplasties January 1996–November 2008 in Geneva University Hospitals. Retrospective documentation of remote infections occurring in hospitalised patients after arthroplasty.
Results: A total of 6101 episodes with 4002 hip (66%) and 2099 knee arthroplasties (34%) were retrieved. Among 71 infections, 64 (90%) were surgical site infections. Seven (total incidence 7/6101, 0.1%) were secondary to remote infections. Secondary infections occurred later post-surgery (on average 19 months vs. 46 months, Wilcoxon-rank sum test, p = 0.024); six of them even 24 months post-arthroplasty. The cohort patients faced 553 infections occurring after a median delay of 33 months post-arthroplasty, 61% after 24 months. In 23 episodes of remote infections (23/553, 4%) the patients were in septic shock and 25 patients died because of remote infection (5%). 39 episodes had an abscess and 81 were bacteraemic. None of them developed secondary arthroplasty infection. The ratio of secondary infections to potential exposure was 1:79. In multivariate analysis, a high Body Mass Index (OR 1.1, 95% CI 1.01–1.1) and revision arthroplasty (OR 2.7, 95% CI 1.1–6.9) were significantly associated with arthroplasty infections. There were no specific parameters detected for secondary infection.
Conclusion: Secondary arthroplasty infections are rare compared to the exposure the patients face after initial surgery. Infections and exposure occur both mostly after 24 months post-arthroplasty.

P134

Enterococcal bacteremia in organ transplant recipients – comparison with non-transplant patients

Von Both U., Walti R., Ruef C.
University Hospital Zurich, Infectious Diseases and Hospital Epidemiology, Zurich, Switzerland

Objective: To describe the clinical and microbiological features of enterococcal bacteremia among organ transplant recipients in a Swiss university hospital.
Methods: Retrospective analysis of cases of enterococcal bacteremia (n = 88) among in-patients based on positive blood cultures during 2006 and 2007. Assessment of presence of potential risk factors for bacteremia (underlying diseases, surgical procedures, ICU stay, exposure to antibiotics), treatment and outcome of bacteremia based on medical records.
Results: Enterococcal bacteremia was diagnosed in 18 organ recipients (14 male, median age 53.5 years, range: 17–74; 6 liver, 4 kidney, 3 lung, 2 heart, 1 heart/lung, 2 bone marrow or stem cell transplant recipients; 7 transplants were received during the same admission as the bacteremia occurred) and 70 non-transplant patients. E. faecium was responsible for 83.3% of bacteremia episodes of transplant patients, but only for 40% of episodes in non-transplant patients (p = 0.002, OR 8.46, CI95 2.23–32.02). Primary bacteremia was the cause of the infection among 38.9% of transplant cases, and 67.4% of non-transplant cases, respectively (p = 0.023; OR 0.31, CI95 0.11–0.88). Both, surgical site infections and intraabdominal infections were significantly more often identified as origin of enterococcal bacteremia in transplant recipients than in non-transplant patients (surgical site infections: 22.2% versus 5.8%, p = 0.024, OR 4.63, CI95 1.1–19.4; intraabdominal infections: 22.2% versus 5.8%, p = 0.024, OR 4.63, CI951.1–19.4). Exposure to antibiotics prior to bacteremia was longer (mean 20.4 days) among transplant patients than non-transplant patients (mean 15.4 days). Outcome of bacteremia was favourable among transplant recipients (cure rate 94.4% versus 76.7% in non-transplant patients, p = 0.093, OR 5.88, CI95 0.73–47.4).
Conclusions: Enterococcal bacteremia is more likely to be caused by E. faecium among transplant recipients. Surgical site infections and intraabdominal infections are significant risk factors for enterococcal bacteremia in this cohort. Outcome is favourable despite the presence of immunesuppression.

P135

A hypervariable *Staphylococcus epidermidis* strain causing persistent bacteremia with myositis and meningitis after haematogeneic stem cell transplantation

Weisser M., Schoenfelder S. M.K., Orasch C., Arber C., Gratwohl A., Frei R., Flückiger U., Ziebuhr W.
Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Switzerland; Hematology, University Hospital Basel, Switzerland.

Objectives: Microbiological and genetic characterisation of consecutive isolates of Staphylococcus epidermidis (SE) obtained from a severely immunosuppressed patient with a prolonged and fatal infection.
Case and results: A 23-year-old patient developed 3 episodes of sepsis with SE on day 15, 31 and 54 of aplasia after an allogeneic cord blood transplantation for a relapse of a common-B-cell acute lymphoblastic leukemia. Bacteremias recurred despite adequate therapy with vancomycin and several changes of the central venous line. Septic thrombosis and endocarditis were ruled out. Septic manifestations with a bilateral myositis of the tibial muscles with histological necrosis and growth of SE in all biopsies on day 35 and meningitis with 2 small septic lesions in the prefrontal cortex and growth of SE in CSF on day 57 occurred. After 60 days of non-engraftment a haplo-identical rescue transplantation from the mother

was performed and granulocyte transfusions were given. On day 74 of aplasia the patient died of a rapidly progressive acute respiratory distress syndrome and multiorgan failure. In autopsy no signs of infection could be documented. 12 isolates obtained from 6 consecutive samples (5 blood cultures (BC) and 1 cerebrospinal fluid (CSF)) were identified as SE by standard microbiological techniques and 16S rRNA gene sequencing. Resistance pattern was uniform except for increasing resistance to oxacillin over time. Oxacillin-susceptible isolates were mecA gene-negative, biofilm-negative and lacked PIA-mediating ica genes. Oxacillin-resistant isolates were mecA gene-positive, increasingly biofilm-producing and had PIA-mediating ica genes. DNA fingerprinting by PFGE demonstrated the clonal origin of the isolates and MLST analysis revealed the ST27 or alternatively ST2 sequence type, known as a nosocomial epidemic strain. Ica-positive variants exhibited 5 IS256-specific bands whereas ica-negative isolates displayed only 4.
Conclusion: The icaADBC-positive ST27/ST2 clone identified in this severely immunosuppressed patient suffering from prolonged and ultimately fatal infection, achieves pathogenicity by multiresistance, increasing production of biofilm and genetic flexibility.

Posters – Basic infectious diseases

P136

P136: replaced by a short communication in symposium 9.

Polymorphisms in toll-like receptor 4 and invasive aspergillosis in hematopoietic cells transplantation

Bochud P.-Y., Chien J.-W., Marr K.-A., Leisenring W., Upton A., Janer M., Rodrigues S.D., Li S., Hansen J.-A ., Zhao L.-P., Aderem A., Boeckh M.
UNIL/CHUV, Microbiology and Infectious Diseases, Lausanne, Switzerland

Invasive aspergillosis (IA) has become increasingly frequent among allogenic hematopoietic cell transplant recipients (HCT), with an incidence rate of up to 12%. Despite the availability of new azoles and echinochandins antifungal drugs, the outcome remains poor, with a 1-year mortality of 50–80%, making IA one of the leading infection-related causes of death among allogenic HCT patients. Common polymorphisms in Toll-like receptors genes have been associated with susceptibility to several infections. We hypothesized that single nucleotide polymorphisms (SNPs) in TLRs influence susceptibility to IA in HCT recipients.
Methods: Twenty single nucleotide polymorphisms (SNPs) in TLRs 2,3,4 and 9 were analyzed in a cohort of 336 HCT recipients and their unrelated donors, using a mass array genotyping platform (Sequenom). Risk of IA was assessed in multivariate Cox regression analysis. The analyses were replicated in a validation study of 103 cases and 263 matched controls of HCT from related/unrelated donors.
Results: In the discovery study, two donor TLR4 haplotypes (S3/S4) increased the risk of IA (adjusted HR = 2.20 [1.14–4.25], P = 0.02 for S3, and HR = 6.16 [1.97–19.26], P = 0.002 for S4). Haplotype S4 included carriers of 2 SNPs in strong linkage disequilibrium (1063 A/G [D299G] and 1363 C/T [T399I]) that influence TLR4 function. In the validation study, donor S4 also increased the risk of IA (OR = 2.63 [1.19–5.84], P = 0.02); the association was present in unrelated (OR = 5.00 [1.04–24.01], P = 0.04) but it was borderline for related (OR = 2.29 [0.93–5.68], P = 0.07) HCT recipients. In the discovery study, positive donor/recipient CMV serology (CMV+) and/or donor S4 (S4+) increased 3-year probability of IA from 1% to 12% (P = 0.02) and non-relapse death from 22% to 35% (P = 0.02), compared to CMV-/S4-.
Conclusion: This study suggests an association between donor TLR4 haplotype S4 and the risk of IA in HCT recipients from unrelated donors.

P137

Genotype 3 is associated with rapid histological progression in chronic HCV infection

Bochud P.-Y., Overbeck K., Bochud M., Rickenbach M., Dufour J.-F, Muellhaupt B., Borovicka J., Heim M., Moradpour D., Cerny A., Malinverni R., Francioli P., Negro F. and the Swiss Hepatitis C Cohort Study
UNIL/CHUV, Infectious Diseases & Microbiology, Lausanne-CHUV, Switzerland

While several risk factors for the histological progression of chronic hepatitis C have been identified, the contribution of HCV genotypes to liver fibrosis evolution remains controversial. The aim of the present study was to assess independent predictors for fibrosis progression.
Methods: We identified 1540 patients from the Swiss Hepatitis C Cohort database with at least one liver biopsy prior to antiviral treatment. Factors associated with fibrosis stage, steatosis and histological activity were assessed in univariate and multivariate regression models. Fibrosis progression rate per year was calculated in a subgroup of 1263 patients, in whom risk factors were assessed by cumulative incidence curves, logistic and linear regression models.
Results: Independent risk factors for rapid fibrosis progression included male sex (OR = 1.66, 95% CI 1.25–2.21, P <0.001), age at infection (OR = 1.08, 95% CI 1.06–1.10, P <0.001), histological activity (OR = 2.14, 95% CI 1.61–2.85, P <0.001) and genotype 3 (OR = 1.97, 95% CI 1.43–2.72, P <0.001). Genotype 2 was associated with slow progression (OR = 0.51, 95% CI 0.30–0.89, P = 0.02), but this observation may be due to the decreased prevalence of genotype 2 over the last decades, leading to an overrepresentation of subjects with genotype 2 with a slow progression rate.
Conclusion: This study shows a significant association of genotype 3 with accelerated fibrosis. While assessing risk factors for fibrosis progression, the changing epidemiology of HCV genotypes over time needs to be taken into account.

P138

Molecular diagnosis of *Kingella kingae* osteoarticular infections by specific real-time PCR assay

Cherkaoui A., Ceroni D., Emonet S., Lefevre Y., Schrenzel J.
Hôpitaux universitaires de Genève, Laboratoire central de Bactériologie, Genève, Switzerland

Kingella kingae is an emerging pathogen that is recognized as a causative agent of septic arthritis and osteomyelitis, primarily in infants and children. The bacterium is best detected by rapid inoculation in blood culture systems or by real-time PCR assays. Pathogenesis of the agent was linked recently to the production of a potent cytotoxin, known as RTX, which is toxic to a variety of human cell types. The locus encoding the RTX toxin is thought to be a putative virulence factor, and is, apparently, essential for inducing cytotoxic effects on respiratory epithelial, synovial and macrophage-like cells. Herein, we describe a novel real-time PCR assay that targets the RTX toxin gene and illustrate its use in two clinical cases. The assay exhibited a sensitivity of 30 c.f.u., which is 10-fold more sensitive than a previously published semi-nested broad-range 16S rRNA gene PCR, and showed no cross-reactivity with several related species and common osteoarticular pathogens.

P139

Development of a modified broad-range 16S rDNA PCR for the diagnostic in clinical microbiology

Cherkaoui A., Emonet S., Candolfi B., Schrenzel J.
Hôpitaux universitaires de Genève, Laboratoire central de Bactériologie, Genève, Switzerland

Broad-range PCR followed by sequencing has been successfully developed to identify microorganisms involved in infections when patients have previously received antibiotics or for detecting fastidious organisms. The major obstacle for broad-range PCR amplification is the presence of bacterial DNA in the Taq DNA polymerase and laboratory reagents, including some of the highest nominal purity. In this regard, it should be noted that a broad-range bacterial PCR is exquisitely susceptible to interference by bacterial targets of any origin, not only those from previous amplifications (as all PCR-based methods). The contaminating DNA is effectively amplified, giving rise to false-positive results. Thus, achieving high sensitivity is dependent on eliminating such spurious targets, a formidable problem because of the ease with which they can confound the detection of small numbers of molecules. Several investigators have reported methods for reducing or eliminating the amplification of contaminating DNA sequences in Taq DNA polymerase, including the use of Sau3AI restriction endonuclease, DNase I, 8-MOP treatment, ultrafiltration of the PCR mix (using the Amicon Microcon YM-100 centrifugal filter device) and ultraviolet irradiation. So far, none of these methods has been shown to be entirely effective or reproducible. We describe a method that uses an Exonuclease III to destroy the ability of contaminating sequences to act as templates in a broad-range PCR that uses 16S rRNA gene-based primers. We detail its development and report its use in several clinical cases of invasive infectious diseases.

P140

Combination of daptomycin with ampicillin prevents the emergence of daptomycin-resistant enterococci in vitro

Entenza J. M., Giddey M., Vouillamoz J., Majcherczyk P., Moreillon P.
University of Lausanne, Department of Fundamental Microbiology, Biophore Building-UNIL, Lausanne, Switzerland

Daptomycin (DAP) is a lipopeptide active against vancomycin-susceptible (VAN-S) and VAN-resistant (VAN-R) enterococci. DAP-resistant enterococci have been reported from few patients with endocarditis. One strategy to prevent the emergence of drug resistance is the use of antibiotics in combination. We tested the effect of ampicillin (AMP), gentamicin (GEN) or rifampin (RIF) on preventing the selection of DAP-resistant enterococci in vitro.
Methods: Ten E. faecalis (8 VAN-S and 2 VAN-R) and 10 E. faecium (5 VAN-S and 5 VAN-R) were tested for synergy of DAP in combination with AMP, GEN or RIF by Etest method. Resistance studies were performed with two E. faecalis and two E. faecium. Spontaneous resistance to DAP was checked by plating 109 CFU on agar+Ca2+ containing increasing DAP concentrations. For selection of DAP-resistant enterococci and the effect of AMP, GEN or RIF on its prevention, bacteria were stepwise exposed in broth+Ca2+ to increased concentrations of DAP alone or containing a fixed amount (0.25xMIC) of either drug. The MIC was recorded after each cycle.
Results: DAP MICs were 1–2 mg/l. Synergism of DAP with AMP, GEN or RIF was observed in, resp., 10%, 10% and 10% E. faecalis and in 30%, 10% and 40% E. faecium. Antagonism was not detected. No spontaneous DAP-resistant mutants were selected. Sequential exposure of enterococci to DAP alone led to a 2xMIC increase in one strain after 7 cycles and to 8xMIC increase in 3 strains after 5–6 cycles. Addition of AMP avoided DAP-resistance in all strains (2–4x increase of DAP MIC after 7 cycles). GEN and RIF at sub-MIC levels had no effect on preventing DAP-resistance.
Conclusions: DAP-resistant enterococci were only obtained by serial passage on DAP. Combining DAP with AMP prevented the selection of DAP-resistant enterococci. This combination is a promising option to prevent DAP resistance in difficult-to-treat enteroccocal infections.

P141

Combination of daptomycin with amoxicillin/clavulanate prevents the selection of daptomycin-resistant *Staphylococcus aureus in vitro*

Entenza J. M., Vouillamoz J., Giddey M., Majcherczyk P., Moreillon P.
University of Lausanne, Department of Fundamental Microbiology, Biophore Building-UNIL, Lausanne, Switzerland

Daptomycin (DAP) is a lipopeptide with potent activity against S. aureus including MRSA and GISA strains. However, DAP efficacy against S. aureus infections might be jeopardized by the isolation of non-susceptible or resistant strains. One strategy to prevent the emergence of drug resistance is the use of antibiotics in combination. We tested the effect of amoxicillin/clavulanate (AMC), gentamicin (GEN) or rifampin (RIF) on preventing selection of DAP-resistant S. aureus in vitro.
Methods: Sixteen MRSA and 3 GISA were first screened for synergy of DAP in combination with AMC, GEN or RIF by Etest method. Resistance studies were then performed with 4 isolates (3 MRSA and 1 GISA). Spontaneous resistance to DAP was checked by plating 109 CFU on agar+Ca2+ containing increasing concentrations of DAP. To assess for selection of DAP-resistant S. aureus and the effect of AMC, GEN or RIF on its prevention, bacteria were stepwise exposed in broth+Ca2+ to increased concentrations of DAP alone or containing a fixed amount (0.25xMIC) of the partner drug. The MIC was recorded after each cycle.
Results: MICs of DAP were 0.5–2 mg/l. DAP in combination with AMC, GEN or RIF was synergistic against 26%, 21% and 21% isolates, resp. Antagonism was not detected. No spontaneous DAP-resistant mutants were found. Sequential exposure of S. aureus to DAP alone led to an 8xMIC increase after 4–7 cycles. Addition of AMC prevented DAP-resistance (2–4x increase of DAP MIC after 7 cycles). At sub-MIC concentrations, GEN had no effect on delaying DAP-resistance. RIF avoided it (2x increase of DAP MIC after 7 cycles) in only 1 isolate.
Conclusions: DAP-resistant S. aureus were only obtained by serial passage of the organisms on DAP. Combining DAP with AMC prevented the selection of DAP-resistant S. aureus. Whether this combination can suppress DAP-resistant S. aureus in vivo deserves further examination.

P142

The role of PD-1 in chronic LCMV infection

Frebel H., Oxenius A.
Institute of Microbiology, ETHZ, Biology, Zurich, Switzerland

Though the immune system is capable of clearing most types of viral infections, there are numerous viral pathogens that cause infections being slowly or never resolved and therefore leading to a protracted or chronic infection of the host. While the causes of the immune system's inability to resolve distinct viral infections still are matter of intense investigation, it is known that endogenous immunoregulatory mechanisms can contribute to the failure of the immune system to control these infections. By infecting mice with the arenavirus LCMV (Lymphocytic choriomeningitis virus), a resolved or chronic infection can be induced, depending on the respective viral strain and the dose of injection. Chronic LCMV infection is marked by the functional exhaustion of CD8 and CD4 T cells which is characterized by a strong reduction of cytokine secretion (IL-2, TNFα and IFNγ), a reduced capability to proliferate and an impaired development of T cell memory. As T cell responses are pivotal for the control of LCMV infection, the observation of functional exhaustion of T cells in chronic infection might be intimately linked to the failure of antiviral control. This study aims at investigating the impact of different immunoregulatory pathways on the development and maintenance of T cell exhaustion in chronic LCMV infection with a special focus on the inhibitory PD-1/PD-L1 pathway. An LCMV infection which normally leads to chronicity in wt mice is lethal in PD-1 ko mice within 7 days. The question is addressed which cellular mechanisms account for this lethality. Preliminary data suggest an essential role of CD8 T cells, as antibody-mediated depletion prior to infection results in a prolonged survival. However, on the day of decease, the frequency and the functionaltiy of LCMV-specific CD8 T cells in chronically infected PD-1 ko mice is comparable to that in wild type mice, questioning a direct CD8 T cell-mediated immunopathology.

P143

The role of AliB homologues in the capsule operon of *Streptococcus pneumoniae* in bacterial growth and colonization of the nasopharynx

Hathaway L.J., Bättig P., Aebi S., Kadioglu A., Mühlemann K.
Institute for Infectious Diseases, Bern, Switzerland

Nonencapsulated S. pneumoniae may act as genetic reservoirs, e.g. for antibiotic resistance, and may be on the evolutionary journey towards commensalism. AliB-like ORF 1 and ORF 2 (homologues of aliB of Ami-AliA/AliB permease) are found in the capsule operon of some nonencapsulated clinical isolates. We hypothesize that they encode colonization factors shared with commensal bacteria.
Methods: Expression of the aliB-like ORFs was determined by real-time PCR. Bacterial growth in culture (OD600nm) was measured for a clinical strain with the aliB-like ORFs and its mutants lacking them and for a backtransformant in which they were restored. To assess nasopharyngeal colonization, bacteria were administered into the nostrils of mice under anaesthesia and the colony forming units in nasopharyngeal homogenates at various timepoints after inoculation counted.
Results: Both aliB-like ORFs are expressed in vitro with expression of AliB-like ORF 1 being 1.7 to 3.2 fold greater than that of ORF 2. AliB-like ORF 1 gives a growth advantage in dilute (1:1 Cden: brain heart infusion broth (BHI)), but not rich (BHI with 5% serum), medium in vitro by reducing the lag phase of growth. In the murine model, a nonencapsulated clinical isolate has an advantage in nasopharyngeal colonization in the 24 hours post-inoculation over its mutant lacking aliB-like ORF 1 and ORF 2. BLAST analysis reveals that aliB-like ORF 1 and ORF 2 are also found in the capsule operon of some strains of the commensal bacteria S. oralis and S. mitis. The entire aliB-like ORF 2 is found in the capsule operon of pneumococcal serotypes 25a, 25f and 38 and a fragment in 66 other serotypes.
Conclusions: We propose that aliB-like ORF 1 confers a growth advantage in nutrient-restricted situations such as the nasopharynx and so acts as a colonization factor giving a selective advantage to strains which possess it.

P144

Moraxella catarrhalis outer membrane protein M35 – a relevant virulence factor?

Jetter M., Heiniger N., Aebi C.
Institute for Infectious Diseases, Research, Bern, Switzerland

The outer membrane protein M35 is a specific antigenically conserved porin of M. catarrhalis type 1 strains and is involved in the uptake of essential nutrients required for the growth of Moraxella catarrhalis.
Aim: To characterize the role of the outer membrane porin M35 of M. catarrhalis in host-pathogen interactions.
Methods: M35 knockout mutants of the type 1 strains O35E, 300 and 415 were tested for their antimicrobial resistance by E-test. The occurrence of human salivary IgA and serum IgG antibodies against

M35 were investigated by Western Blot. To investigate the degree of conservation of M35 in both phylogenetic subpopulations of M. catarrhalis (type 1 and 2), the M35 gene of type 2 strain 287 was amplified by PCR and sequenced with an AB 3130 Genetic Analyzer.
Results: From a total number of 14 different antibiotics, we observed differences in the MIC (Minimum Inhibitory Concentration) between wild-type and mutant for eight antibiotics. For ampicillin and amoxicillin-clavulanate we observed a two to four fold higher MIC in the M35 mutants than in the wild-type strains. Western Blot analysis demonstrated that both human saliva and serum contain anti-M35 IgA and IgG, respectively. Sequencing analysis of M35 of type 2 strain 287 showed a 94.2% identity on the DNA level and 92.8% identity on the amino acid level in comparison with type 1 strains. The structural analysis of the protein sequence suggested that this diversity is unlikely to result in functional differences.
Conclusion: The increase of the ampicillin and amoxicillin-clavulanate MIC in the M35 knock-out mutants suggests that this porin affects the outer membrane permeability for aminopenicillins. The presence of IgA and IgG antibodies in healthy human donors indicates that M35 is not only a possible virulence factor but also a relevant antigen and – in connection with this and its high conservation within the different strains – a possible vaccine candidate.

P145

Daptomycin alone and in combination with rifampin for the treatment of experimental methicillin-resistant *Staphylococcus aureus* (MRSA) implant-associated infection

John A.-K., Baldoni D., Rajacic Z., Zimmerli W., Trampuz A.
University Hospital Basel, Department of Biomedicine, Basel, Switzerland

We investigated the in-vitro susceptibility, pharmacokinetics and treatment efficacy of daptomycin, alone and in combination with rifampin, compared to vancomycin, linezolid and levofloxacin, in a MRSA foreign-body infection model.
Methods: Daptomycin MIC and MBC values in logarithmic (MBClog) and stationary growth phase (MBCstat) for MRSA (ATCC 43300) were determined in growth media supplemented with 50 mg/l Ca2+. For pharmacokinetics and treatment studies, the guinea pig infection model with subcutaneously implanted Teflon cages was used. After 3 days of cage-infection with MRSA, intraperitoneal treatment was started for 4 days. Bacteria were determined in cage fluid 5 days after treatment; cages were then explanted and cultured to detect adherent bacteria and evaluate development of rifampin-resistance.
Results: The daptomycin MIC and MBClog and MBCstat were 0.625, 0.625 and 20 µg/ml, respectively. In time-kill studies in the stationary growth phase, daptomycin showed rapid and concentration-dependent killing of MRSA. At concentrations above 20 µg/ml, daptomycin reduced >3 log CFU/ml in 2 to 4 h. After a single intraperitoneal dose of 20, 30 and 40 mg/kg, daptomycin reached peak concentrations in sterile cage fluid of 23, 46 and 54 µg/ml, respectively, at 4–6 h after dosing. Daptomycin alone reduced planktonic MRSA by <0.5 log CFU/ml, whereas in combination with rifampin, planktonic MRSA were reduced by >6 log CFU/ml. In combination with rifampin, daptomycin showed a cure rate of 25% (at 20 mg/kg) and 67% (at 30 mg/kg), vancomycin of 8%, linezolid of 0% and levofloxacin of 58%. In combination with rifampin, no rifampin-resistance developed in adherent MRSA with levofloxacin and daptomycin at 30 mg/kg, whereas 8%, 17% and 58% rifampin-resistance developed with linezolid, daptomycin at 20 mg/kg and vancomycin, respectively.
Conclusions: The combination of daptomycin in a high dose with rifampin is a promising option for treatment of implant-associated MRSA infections.

P146

Schistosoma mansoni TOR: a potential vaccine target

Lochmatter C., Schifferli J.A., Martin P.J.
Department of Biomedicine, Immunonephrology, University Hospital Basel, Basel, Switzerland

A trispanning orphan receptor (TOR) has been described in Schistosoma haematobium and S. mansoni. Here we report the complete molecular organisation of the S. mansoni TOR gene, also known as SmCRIT (complement C2 receptor inhibitor trispanning). SmTOR gene consists of four exons and three introns as shown by cloning the single exons from S. mansoni genomic DNA and the corresponding cDNA from the larval stage (cercaria) and the adult worm. The SmTOR ORF consists of 1260 bp and is longer than previously reported with a fourth transmembrane domain (proposed new name: Tetraspanning Orphan Receptor), with however an unchanged C2 binding domain on the extracellular domain one (ed1). This domain differs in S. japonicum. A protein at the approximate expected molecular weight (55 kDa) was detected in adult worm extracts with polyclonal and monoclonal antibodies. Developmental expression profiling showed SmTOR expression to be highest in cercariae, where we were able to detect it on the surface. The higher expression in cercariae as compared to adult worm is of interest since cercariae are in first contact with human skin. SmTOR might have a function in determining the fate of the infection at this time point. In addition, SmTOR being an antigen at the surface of cercariae may be a good target for vaccine development.

P147

Factors driving memory inflation during mouse cytomegalovirus infection

Mekker A., Häberli L., Karrer U.
Division of Infectious Diseases, University Hospital of Zurich, Zurich, Switzerland

Permanent immune surveillance by virus specific T cells is mandatory for the control of persistent infection with human and mouse Cytomegalovirus (MCMV). Memory inflation, i.e a pattern of longitudinal accumulation of some MCMV-specific CD8+ T cells, is a peculiarity of the T cell response against these persistent viruses. To elucidate the mechanisms contributing to this unusual T cell kinetics we have compared two representative responses longitudinally: M45-specific T cells dominate the acute response and contract thereafter, whereas M38-specific T cells gradually accumulate and become dominant after viral clearance. Upon reinfection of MCMV-immune mice M38-specific T cells expand massively but M45-specific cells do not proliferate despite having a central memory phenotype. However, M45-specific cells do not have an intrinsic proliferative defect, since they vigorously expand after adoptive transfer and secondary challenge in a naïve host. Factors that influence this shift in immunodominance may include T cell competition and a moderate increase in functional avidity of M38-specific cells. Current experiments aim at identifying additional differences in antigen availability and presentation to specific T cells that are likely to be involved in shaping the immunodominance hierarchies and the degree of memory inflation after MCMV-infection.

P148

Dominant negative TNF protects from *Mycobacterium bovis* BCG and endotoxin-induced liver injury without compromising host immunity to mycobacterium tuberculosis and BCG infections

Olleros M., Vesin D., Fotio A., Janssen J.-P., Ryffel B., Rose S., Frémond C., Quesniaux V., Szymkowski D.E., Garcia I.
Faculty of Medicine, University of Geneva, Pathology and Immunology, CMU, Geneva, Switzerland

Tumor necrosis factor (TNF) is associated with the development of inflammatory human pathologies. Antibodies and soluble TNF receptors neutralizing both soluble TNF (solTNF) and transmembrane TNF (tmTNF) are effective treatments for several inflammatory and autoimmune diseases. However, the clinical use of these inhibitors has been linked to serious adverse events, most notably including an increased risk of infections.
Methods: A novel strategy of selective TNF neutralization, consisting of blocking only solTNF while sparing tmTNF, was tested in animal models of Mycobacterium bovis BCG and Mycobacterium tuberculosis infections and acute liver inflammation. To explore the in vivo effects of selectivity for solTNF, we exploited a new class of anti-inflammatory agent known as dominant-negative inhibitors of TNF (DN-TNF).
Results: The present study analyses the effect of a DN-TNF molecule on immune defense against M. bovis BCG and M. tuberculosis infections and protection from hepatitis induced by endotoxin challenge in BCG-infected mice. Inhibition of solTNF by DN-TNF does not affect mycobacterial clearance in infected organs and Th1 type cytokine production and granuloma formation are preserved whereas etanercept (TNFR2-IgG1) treated mice die from M. tuberculosis infection and cytokine production after BCG infection is suppressed. In the same way, DN-TNF efficiently protects from endotoxin-mediated hepatotoxicity in BCG-infected mice reducing serum alanine and aspartate aminotransferase levels, TNF bioactivity, and inflammatory cytokines.
Conclusions: These data show that selective inhibition of solTNF with DN-TNF efficiently protects from acute liver inflammatory reactions, yet does not suppress immunity to mycobacterial infections. These findings may be relevant to the development of novel therapeutic strategies in inflammatory liver diseases that do not compromise host immunity.

P149

Liquid chromatography tandem mass spectrometry (LC-MS/MS) and bioassay with extended analytical range for measurement of posaconazole (POS) plasma levels

Pascual A., Rochat B., Pesse B., Sanglard D., Bille J., Calandra T., Marchetti O.
Infectious Diseases, Pediatrics, Lausanne, Switzerland

Background: POS is a new generation broad-spectrum azole antifungal agent. The large variability of POS plasma levels may influence efficacy in patients with life-threatening invasive mycoses

(Walsh CID 2007). LC-MS/MS is the reference analytical method for measurement of drugs plasma levels, whereas bioassay is easy to perform and inexpensive. The aim of the study was to develop and cross-validate over the POS plasma concentration range in patients a LC-MS/MS method and a bioassay using a POS hypersusceptible mutant.
Methods: POS extraction from plasma by protein precipitation with MeCN/MeOH. LC-MS/MS: reverse phase separation with a C18 column, electrospray ionization. Bioassay: *C. albicans* DSY2621 (Δcdr1, Δcdr2, Δflu, Δmdr1, Δcna), MIC POS 0.002 mg/L; agar diffusion in YNB. Validation according to international guidelines (FDA, 2001). Accuracy: measured/nominal value x 100. Precision: SD/mean measured values x 100. Intra-run (n = 5) and inter-run (n = 5) validations were performed with quality controls ranging from 0.05 to 7.5 mg/L.
Results: Reproducible standard curves were obtained with both methods (r ≥0.99). Analytical performances are summarized in the table:

	LC-MS/MS	Bioassay
Analytical range (mg/L)	0.014–12	0.028–12
Accuracy intra-/inter-run, mean % ± SD	106.1 ± 2.3 / 103 ± 3.7	102.2 ± 5.6 / 104 ± 1
Precision intra-/inter-run, mean % ± SD	7.4 ± 2 / 6.9 ± 3.3	5.5 ± 2.7 / 4.2 ± 1.3

POS values were stable (± 15% of nominal value) in plasma and whole blood stored during 4 d at 4 and 21°C, and in plasma stored during 3 months at –80 °C or after 4 freeze-thaw cycles. Comparison of POS levels measured by LC-MS/MS and bioassay in 25 clinical samples showed a high concordance (r^2 x a = 0.96).
Conclusions: Robust LC-MS/MS and bioassay methods with extended analytical range have been developed and cross-validated for the measurement of posaconazole plasma levels.

P150

B cells help to maintain functionality of inflating CD8+ T cells during cytomegalovirus infection

Tchang V., Mekker A., Häberli I., Karrer U.
Infectious Diseases and Hospital Epidemiology, Department of Internal Medicine, Zurich, Switzerland

Infection with Cytomegalovirus (CMV) leads to life long viral carriage with the need for constant immune surveillance mainly by T cells and NK cells. B cells and neutralising antibodies seem to be less critical apart from some containment of viral spread during primary infection and reactivation. During persistent infection, CMV-specific T cells slowly accumulate leading to large populations of up to 50% of total memory CD8+ T cells in ageing hosts. This has fostered the hypothesis that CMV-infection may increasingly restrict the overall T cell diversity in the elderly and thus contribute to immune senescence. Although antigen availability during CMV-reactivation is likely to be involved, the crucial factors responsible for memory inflation have not yet been elucidated. Here we tested whether memory inflation after mouse (M)CMV infection is influenced by increased viral dissemination in the absence of B cells and antibodies. In B cell-deficient μMT and JHT mice viral clearance was delayed in lung and spleen after primary MCMV-infection but productive viral replication was nevertheless terminated within 60 days. CD8+ T cell responses specific for inflating (M38) and non-inflating (M45) MCMV epitopes were delayed and their functionality concerning secretion of effector cytokines was reduced. Whereas the degree of memory inflation was not significantly altered in B cell deficient mice, the function of accumulating T cells gradually deteriorated over time without evidence for loss of viral control. These results suggest that B cells and antibodies help to limit the functional exhaustion of MCMV-specific CD8+ T cells and thus contribute to the long term control of MCMV-infection. In addition, they may be involved in the prevention of CMV-induced immune senescence.

P151

In vitro activity of gallium maltolate against methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* and *S. epidermidis*

Trampuz A. Baldoni D., Steinhuber A., Zimmerli W.
University Hospital Basel, Department of Biomedicine, Basel, Switzerland

Ga(III) is a semi-metallic element physically similar to Fe(III) and thus thought to compete for iron-binding sites of transporters and enzymes. We investigated the in vitro anti-staphylococcal activity of gallium maltolate (GaM), an oral gallium formulation.
Methods: The following strains were tested: Staphylococcus aureus ATCC 29213 (MSSA), S. aureus ATCC 43300 (MRSA), S. epidermidis 1457 (MSSE) and S. epidermidis B3972 (MRSE). Susceptibility towards GaM was assessed by a broth macrodilution assay in an iron-limited medium RPMI for determination of MIC and MBClog, whereas PBS was used for stationary MBCstat. The anti-biofilm activity of GaM was investigated with a 96-well microtiter plate assay to determine the minimal biofilm inhibitory concentration (MBIC). Killing and inhibition profiles were investigated by time-kill studies and heat measurements (calorimetry).
Results: GaM MIC/MBClog/MBCstat/MBIC values were (mg/L): 1500/ >6000/ >6000/ >6000 (MSSA) and 1000/ 6000/ >6000/ 6000 (MRSA), 100/ 1500/ 4500/ 200 (MSSE) and 200/ 1500/ 1500/ 560 (MRSE). In time-kill studies and in calorimetric assay, GaM displayed a time-dependent activity at inhibitory concentrations and both time- and dose-dependent activity at sub-inhibitory concentrations.
Conclusion: GaM was bactericidal against S. epidermidis and MRSA, whereas only bacteriostatic activity was noted against MSSA. The anti-staphylococcal activity was exhibited at high GaM concentrations, 100× to 1000× higher than the serum concentrations achieved in healthy volunteers after a single oral administration of GaM. However, high local GaM concentrations may be achieved after topical use, e.g. for staphylococcal decolonization of mucosa or skin or for coating of implants.

Posters – HIV

P152

The TLR4 D299G SNP influences susceptibility to opportunistic infections (OIS) in the Swiss HIV cohort study (SHCS)

Cai T., Zhang K., Fellay J., Rickenbach M., Calandra T., Telenti A., Bochud P.-Y. and the Swiss HIV Cohort Study (SHCS)
CHUV, the Institute of Microbiology, Institute of Microbiology, Lausanne, Switzerland

While some HIV+ patients survive for extended periods of time with low CD4 counts, others under similar conditions rapidly develop OIs. Single nucleotide polymorphisms in TLRs have been associated with susceptibility to infections. We hypothesized the TLR4 D299G SNP (one of the most relevant TLR SNP) influences susceptibility to OIs in SHCS patients.
Methods: Among 6271 Caucasian patients with available DNA and genetic consent, 1585 have been previously genotyped for TLR4 D299G. Time below specific CD4 cells limits (<400, <200, <100 or <50, depending of the type of OI) was assessed by individual CD4 cells curve smoothing using the locpoly program (Stata). The association between D299G and OIs was assessed using Poisson regression models, after adjustment for age, sex, infection risk factors and year of SHCS entry.
Results: Among 1368 patients with CD4<400, 128 had severe candidiasis (mainly candida oesophagitis), 98 had VZV infection (e.g. multidermatoma or relapsing zona), 44 had HSV infection (mucocutaneous ulceration or HSV disease) and 16 had tuberculosis. Among 521 patients with CD4<200, 66 had Pneumocysitis jirovecii pneumonia (PCP) and 27 had toxoplasmosis (e.g. cerebral). Finally, among 303 patients with CD4<100, 37 had CMV infection (CMV disease or retinitis). The D299G SNP was associated with toxoplasmosis (Incidence Rate Ratio [IRR] = 2.4 (95% CI 1.6–3.7), P = 0.041), PCP (IRR = 1.95 [1.4-2.7], P = 0.041), and tended to be associated with tuberculosis (IRR = 2.6 [1.5-4.3], P = 0.077), but no association was found for CMV infection (P = 0.3), severe candidiasis (P = 0.7), HSV (P = 1.0) and VZV (P = 0.3).
Conclusion: The TLR4 D299G SNP may influence susceptibility to several OIs independently of CD4 levels. The SHCS is a large cohort of HIV patient that will provide a unique opportunity to increase statistical power and study the role of innate immune gene polymorphisms on susceptibility to several OIs.

P153

Parvovirus B19 and erythrovirus type 2 and 3 infections are infrequent in HIV-infected individuals with chronic anemia

Calmy A.. Ferry T., Hirschel B., Dang T., Meylan P., Rauch A., Weber R., Elzi L., Bernasconi E., Vernazza P. and the SHCS
Hôpitaux Universitaires de Genève, Unité VIH/SIDA, Geneva, Switzerland

Erythroviruses, Parvovirus B19 (PVB19) and the newly described Erythrovirus genotype 2 and 3, mainly target human erythroid progenitors. The pattern of clinical disease due to PVB19 infection is strongly influenced by the hematologic and immunologic status of the

host. In immunosuppressed patients, a reactivation of PVB19 may occur and may lead to severe acute or chronic anemia and zidovudine (AZT) intolerance. The pathogenic role of newly described Erythroviruses is unknown.
Objectives: Screening for Erythrovirus replication in a large cohort of HIV-infected patients presenting with CD4 cell count <500 cells/mm^3 and chronic anemia to test if patients with HIV infection were at risk for symptomatic parvovirus infection.
Methods: Patients included in the Swiss HIV Cohort study from 1998 to 2007 were selected according to the following criteria: (i) persistent anemia (hemoglobin [Hb] level below 10.5 g/dL during at least three months) (ii) CD4 cell count <500 cells/mm^3 during the episode of anemia and (iii) availability of at least one frozen serum or plasma sample during the period of anemia. Detection and quantification of Erythroviruses was performed with a real-time PCR targeting the VP1 gene, a conserved region of the Erythrovirus genomes.
Results: 428 patients were included in the study (median age 44 years, female sex 61%, intravenous drug user 36%, median CD4 cell count 187 cells/mm^3, median Hb level 9.5 g/dL, AZT exposure 41%); circulating Erythrovirus DNA was detected in 16 of them. Viral load ranged from 18 to 6820 copies/mL and was low (<500 copies/mL) in 13 patients. No differences were noticed after comparison of patients with or without Erythrovirus replication with regards to route of transmission, CD4 cell count and AZT exposure.
Conclusion: Erythtroviruses infections appear to be an infrequent finding in HIV-infected patients presenting with low CD4 cell count and chronic anemia, despite the use of an ultrasensitive PCR technique.

P154

Low sensitivity of an Interferon-γ releasing assay (Elispot-TB™) for the diagnosis of latent tuberculosis in HIV-infected individuals

Elzi L., Steffen I., Furrer H., Bassetti S., Battegay M. and the Swiss HIV Cohort Study.
Klinik für Infektiologie & Spitalhygiene, Universitätsspital Basel, Basel, Switzerland

Interferon-γ release assays (IFNγ) are replacing the tuberculin skin test (TST) for diagnosis of Mycobacterium tuberculosis infection. Data on sensitivity are controversial in the setting of immunosuppression.
Methods: HIV-infected individuals who developed culture-confirmed tuberculosis after inclusion in the Swiss HIV Cohort Study were analyzed. IFNγ assay (Elispot-TB™) was performed from lymphocytes stored within 6 months before active tuberculosis was diagnosed.
Results: 64 HIV-infected individuals (68.8% males, 54.3% of non-white ethnicity, median age 35 (IQR 31–42) years, 18% with prior AIDS) were analyzed. The median CD4 T-count was 223 cells/µl (IQR 103–339), HIV-RNA was 4.7 log10 copies/ml (IQR 4.3–5.3). The IFNγ assay was performed using lymphocytes obtained at a median of 91 days (IQR 47–167) before active tuberculosis. Elispot-TB™ resulted positive in 25 patients (39.1%), negative in 18 (28.1%) and indeterminate in 21 (32.8%), corresponding to a sensitivity of 58% (95% CI 43-74%) if indeterminate results were excluded. Sensitivity of IFNγ test was independent of CD4 T-count (p = 0.698). Among 44 individuals with available TST results, 22 (50%) had a positive TST, defined as a skin induration of >5 mm. Agreement between TST and Elispot-TB™ was noted in only 56.8% (kappa = 0.14, p = 0.177). In subjects with positive test results by either TST or Elispot-TB™, only 34.5% (10/29) had positive results with both modalities. TST-positive/IFNγ negative discordant results were noted in 27.2% of subjects and TST-negative/IFNγ positive results in 15.9%. If TST and IFNγ assay were combined (at least one test positive) a sensitivity of 66% (95%CI 51–80%) could be reached. In the multivariate analysis, age was the only risk factor of having both tests negative (OR 3.15, 95%CI 1.23–8.1, p = 0.017, per 10 years older).
Conclusions: IFNγ assay (Elispot-TB™) alone has a low sensitivity to detect latent tuberculosis in HIV-infected individuals. Combination of TST and IFNγ test may be useful in clinical practice.

P155

A simple and smart tool to start combined antiretroviral therapy (cART) successfully

Fehr J., Nicca D., Spirig R., Langewitz W., Haerry D., Battegay M.
University Hospital of Basel, Infectious Diseases and Hospital Hygiene, Basel, Switzerland

Recent treatment guidelines support the trend to start cART earlier i.e. at CD4- cell count of 350 cells/ul. Treatment success of cART depends on the readiness of patients to assume an active role in their every day medication management. Willingness to engage in cART marks the end of a decision-making process. There is currently a lack of structured tools to optimally support patients in their decision making process and to provide best guidance towards a successful start of cART.
Objective: To create a simple and easy to use tool to provide best support for patients in the situation of initiation of cART.
Methods: Based on literature review, on own quantitative and qualitative study and on expert-discussions we developed a readiness concept which was integrated into daily clinical work enabling the development of a readiness tool.
Results: The underlying concept takes into account that patients arriving at the clinic may be at different stages of readiness: Precontemplation, contemplation and preparation. Providers have to assess their patients' stages of readiness before therapy start and then to support and intervene according to the stage found. Furthermore, patients should be screened for decision-making and adherence barriers i.e. patients' related factors (depression, harmful alcohol or recreational drug use, cognitive problems, low health literacy) and system related factors (health insurance and drug supply, continuity of drug supply, social support and disclosure). All these factors have been integrated and condensed to an algorithm (http://www.eacs.eu/guide/1_Treatment_of_HIV_Infected_Adults.pdf).
Conclusion: We were able to develop an algorithm to assess and support patients' readiness to start cART which is easy to implement into daily practice and which provides a basis for the successful initiation of cART and long-term adherence to the treatment.

P156

Pre-treatment levels of innate and adaptive LPS-scavengers are inversely correlated with CD4+ T-cell recovery after initiation of ART

Gasser O., Wolbers M., Battegay M., Brander C., Hess C. and the Swiss HIV Cohort Study
University Hospital Basel, Department Biomedicine, Immunobiology Lab, Basel, Switzerland

Increased "leakiness" of the gastrointestinal mucosa in chronic HIV-infection is associated with bacterial translocation – which can be measured by increased plasma LPS-levels – and results in the induction of LPS-mediated innate and adaptive immune-mechanisms. The ensuing chronic immune activation has been linked to the pathogenesis of HIV-infection. Little is known on how LPS-levels and LPS-induced immune-mediators are associated with immune recovery in individuals treated with anti-retroviral therapy (ART).
Patients and methods: Soluble CD14 (sCD14), LPS-binding protein (LBP) and endotoxin core antibodies (endoCAb), as well as LPS itself, were quantified in a cohort of 168 HIV-infected individuals prior to initiation of ART. Pre-ART concentrations of these molecules were related to CD4+ T-cell recovery-rates at 6, 12 and 24 months after initiation of ART.
Results: Pre-ART sCD14-levels were associated with ART-mediated CD4+ T-cell recovery at 6 and 12, but not at 24 months after initiation of ART (6 months: r = 0.2072; p = 0.007, 12 months: r = 0.1906; p = 0.01, 24 months: r = 0.1305; p = 0.091). By contrast, a consistent and inverse association of endoCAb levels with ART-mediated CD4+ T-cell recovery was observed (6 months: r = –0.1577; p = 0.041, 12 months: r = –0.2054; p = 0.0076, 24 months: r = –0.2094; p = 0.006). Pre-ART concentrations of LPS and LBP were not linked to CD4+ T-cell recovery.
Conclusion: These data indicate that – in the context of gastro-intestinal mucosa "leakiness"– shedding of sCD14 (reflective of an innate immune mechanism) and production of endoCAb (reflective of an adaptive immune response) differentially relate to the potential for ART-mediated immune-recovery. High levels of endoCAb –plausibly resulting from long-standing exposure to LPS – seem to be reflective of an immune-depletion status difficult to recover from. Increased concentration of sCD14, by contrast, identifies a subset of HIV-infected individuals with a good likelihood for early ART-mediated immune-recovery.

P157

Treatment-dependent loss of polyfunctional CD8+ T-cell responses in HIV-infected kidney transplant recipients is associated with herpesvirus reactivation

Gasser O., Bihl F., Sanghavi S., Rinaldo C., Rowe D, Hess C., Stablein D., Roland M., Stock P., Brander C.
University Hospital Basel, Department Biomedicine, Immunobiology Lab, Basel, Switzerland

Antiretroviral-therapy (ART) has dramatically improved the prognosis of HIV-infection. However, chronic kidney and end-stage renal disease represent major complications for treated HIV-infected (HIV(+)) individuals and HIV(+) patients are becoming more frequently eligible for kidney transplantation. Unfortunately, only scarce data are available on how immunosuppressive strategies relate to transplantation outcome and immune function.
Patients and methods: We determined the impact of transplantation and immune-depleting treatment on CD4+ T-cell counts, HIV-, EBV-, and CMV-viral loads and virus-specific T-cell immunity in a 1-year prospective cohort of 27 HIV(+) kidney transplant recipients. T-cell immunity – i.e. the breadth and magnitude of CD8+ T-cell responses-was assessed by means of IFNγ ELISpot assays targeting specific libraries of virus-derived CD8+ T-cell-restricted epitopes. We also used multi-parameter flow-cytometry to reveal the "poly"-functionality (defined as combined secretion of IFNγ, TNFα and/or IL-2, either as a

combination of 2 or all 3 cytokines) of individual CD8+ T-cell responses.
Results: We show that, over time and across all treatment variations, both the breadth and magnitude of the herpesvirus-specific cytotoxic T-cell (CTL) response increases. However, subsequent analyses revealed a significant depletion of polyfunctional virus-specific CTL in individuals as a result of lymphocyte-depleting thymoglobulin-treatment ($p < 0.05$). Importantly, the overall lack, or decreased magnitude, of polyfunctional EBV-specific CTL was associated with virologic EBV-reactivation events ($p < 0.05$).
Conclusion: The data provide first insights into the immune-reserve in HIV-infected transplant recipients, highlight new immunological effects of thymoglobulin treatment and provide evidence for a direct, a likely causal, link between the absence of specific polyfunctional CTL and loss of viral control. Long-term studies will be needed to assess the clinical risk associated with thymoglobulin treatment, in particular with regards to EBV-associated lymphoproliferative diseases.

P158

Dissociating CD4+ T cell-counts, influenza-specific CD4+ T cell-function and antibody-response in HIV-infected individuals

Hamm S., Mossdorf E., Zenhäusern G., Durovic B., Steffen I., Gasser O., Battegay M., Nüesch R., Hess C.
Unispital Basel, Labor Immunbiologie, Departement Biomedizin, Basel, Switzerland

Infection with influenza remains a potentially fatal threat, particularly for immuno-compromised individuals such as those infected with HIV. Efficient vaccination-programs thus are potentially life saving. CD4+ T cells are thought to be essential in orchestrating the vaccination induced B cell/antibody response. Absolute CD4+ T cell-counts remain the best surrogate for immune-competence in HIV-infected individuals. Little is known on how absolute CD4+ T cell-counts, vaccine-specific CD4+ T cell-function and the antibody-response relate.
Patients and methods: During the vaccination-season '07–'08, PBMC and serum from HIV-negative persons (n = 24), HIV-infected individuals with high (>350/MicroL; n = 21) and HIV-infected individuals with low (<350/MicroL; n = 10) CD4+ T cell-counts, were sampled (pre-vaccine, day 7, 14 and 28 post-vaccine). In each of these three study-populations ELISpot assays were used to enumerate vaccination-induced IFNγ secretion of CD4+ T cells. Cellular "vaccine-responders" were defined as those exhibiting an increase in influenza-specific CD4+ T cells of more than 80 SFC/10^6 PBMC. Influenza-specific antibody-levels were quantified by ELISA.
Results: Post-vaccination median frequencies of IFNγ-secreting CD4+ T cells increased significantly only in HIV-negative and HIV-infected/CD4(high) individuals. The rate of cellular "vaccine-responders" was 87%, 75%, and 66% in HIV-negative, HIV-infected/CD4(high) and HIV-infected/CD4(low) individuals, respectively. By contrast, a statistically significant – and in magnitude comparable – increase in median influenza-specific antibody-levels was observed in all three study-populations.
Discussion: The fact that the percentage of cellular "non-responders" – although steadily decreasing from HIV-infected/CD4(low) to HIV-infected/CD4(high) to HIV-negative individuals – was >10% even among HIV-negative individuals dissociates total CD4+ T cell-counts from the individual vaccine-specific CD4+ T cell-response. This highlights the usefulness of assessing immuno-competence on an individual and pathogen-specific level. Furthermore, the fact that the magnitude of the antibody-response was dissociated from both total and antigen-specific CD4+ T cell-counts rises the question on how well the quality of the humoral response compares among these study-populations.

P159

Determinants of unprotected sex among HIV-infected persons. The Swiss HIV cohort study (SHCS)

Hasse B., Ledergerber B., Jeannin A., Vernazza P., Evison J.-M., Elzi L., Hirschel B., Cavassini M., Bernasconi E., Weber R. and the Swiss HIV Cohort Study
University Hospital Zurich, Clinic of infectious diseases and hospital epid., Zurich, Switzerland

A trend in resurgence of sex without condoms and sexually transmitted infections is observed.
Objectives: To evaluate determinants of unprotected sex with HIV-negative partners or occasional contacts among participants of the SHCS.
Methods: We randomly selected one follow-up visit per patient after 4/2007 and performed a cross-sectional analysis of characteristics for the preceding 6 months.
Results: 4164 of 7111 individuals reported sex with a stable partner (SP) and 359 (8.6%) used condoms incompletely.1663 persons reported sex with an occasional partner (OP) and 268 (16.1%) had unprotected sexual intercourse. Young people tended to use condoms less often, especially in SP ($p < 0.001$). With SP, men who have sex with men (MSM) had unprotected sex only in 4.4% and thus much less than heterosexual men and women (9.8 and 12.5%, $p < 0.001$). With OP, however, groups did not differ ($p = 0.9$). With OP 25.3 % of people without antiretroviral therapy (ART) and 17.1% of people on ART and detectable viral load engaged in unprotected sex, but only 11.5% of treated people with suppressed viremia do so ($p < 0.001$). Alcohol and drug consumption were consistently associated with increased probabilities of unprotected sex, especially i.v. heroin with SP (28.2%) and intranasal Cocaine with OP (31.4%). Persons who report unprotected sex with OP tend to engage in unsafe sex also with their SP ($p < 0.001$). These findings were largely confirmed in multivariable models.
Conclusions: We have identified important determinants of unprotected sex which may inform prevention counseling. However, several aspects (e.g. duration of physician-patient relationship, calendar time) are yet unexplored and interaction terms between variables (e.g. age and gender, transmission category and ART) need to be included. Finally, we have to keep in mind that our analyses are based on self-reported sexual behavior with all its limitations.

P160

Integration of structured counseling for smoking cessation in HIV outpatient care

Huber M., Ledergerber B., Weber R. and the Swiss HIV Cohort Study
University Hospital Zurich, Infectious Diseases, Zurich, Switzerland

HIV-1 infected persons on antiretroviral treatment are at increased risk of cardiovascular disease. Smoking (50–60%) is the most prevalent modifiable risk factor but physicians are often not trained in smoking cessation counseling.
Objectives: To evaluate the uptake of smoking cessation counseling in a single HIV outpatient clinic of the Swiss HIV Cohort Study (SHCS).
Methods: Physicians were subjected to a validated standardized smoking cessation counseling education program. Thereafter they had to complete a short questionnaire on the patients' motivation level to stop smoking and support offered to that respect for every semiannual follow-up visit of participants in the SHCS from 09/2007 until 08/2008. As only few patients contributed multiple visits during this time period, we report results from cross-sectional analyses of individual visits.
Results: Questionnaires were completed for 1448/2001 (72%) visits of 1164 patients. While missing questionnaires were equally distributed between current smokers and non-smokers we observed some heterogeneity with respect to individual physicians. In 690/1448 (48%) visits the patients indicated to currently smoke and 551/690 (80%) were counseled. Reasons for not counseling were other medical priorities (54%), patient's refusal (20%) and lack of time (10%). Motivation levels among counseled smokers were immediate stop of smoking (5%), within the next 6 months (14%), some time later (37%) and no motivation to stop (44%). Physicians could choose single or multiple answers among several options to document the provided support: Short counseling (44%), detailed counseling (13%), agreed upon a date for stopping (3%), agreed upon a next date for discussing stop smoking (7%), provided a handout (21%), referred to specialist (0.7%), nicotine substitution (5%), prescriptions for bupropion hcl (Zyban®) (0.7%) and varenicline (Champix®) (4%).
Conclusions: Structured counseling for smoking cessation is feasible in the HIV outpatient setting. Pharmacotherapy and nicotine substitution were rarely prescribed. Long-term follow-up is needed to evaluate effectiveness.

P161

Association of non-cirrhotic portal hypertension (NCPH) in HIV-infected persons and antiretroviral therapy with didanosine (DDI)

Kovari H., Ledergerber B., Peter U., Flepp M., Jost J., Schmid P., Calmy A., Müllhaupt B., Weber R. and the Swiss HIV Cohort Study
University Hospital Zurich, Division of infectious diseases and hosp. epid., Zurich, Switzerland

Portal hypertension without liver cirrhosis is a newly described life-threatening complication of unknown cause in HIV-infected persons without hepatitis virus co-infections. Postulated pathogenesis includes prolonged exposure to antiretroviral therapy (ART), particularly DDI.
Methods: We performed a nested case control study including 15 patients with cryptogenic non-cirrhotic portal hypertension (NCPH) and 75 matched controls of the Swiss HIV Cohort Study to investigate risk factors for the development of NCPH. Matching criteria were absence of hepatitis virus infection, similar duration of HIV infection, and follow-up to at least the date of diagnosis of NCPH in the respective case.
Results: All 15 cases (13 male; 11 homosexuals, 4 heterosexuals) had endoscopically documented esophageal varices and absence of liver cirrhosis on biopsies. 15 patients had splenomegaly; 7 variceal bleeding; 8 ascites, 5 portal thrombosis; 2 hepatic encephalopathy; 4 died due to hepatic complications. At time of diagnosis of NCPH, no differences in characteristics of cases vs. controls were found in: Median HIV infection date, 04/1990 vs. 01/1990; sex; HIV transmission; ethnicity; duration of follow-up (12.0 vs. 11.9 yrs); CDC disease stage;

peak HIV-RNA; HIV-RNA on ART; plasma lipids, fat loss/accumulation, and blood pressure. Differences were found in: Median age, 52 vs. 43 yrs (conditional logistic regression OR /10 yrs older 2.9 [95% CI 1.4–6.1] p = 0.004); MSM (OR 4.5 [1.2–17] p = 0.03); CD4 count, 197 vs. 522 (OR for CD4 <200, 29.4 [3.6–242] p = 0.002); CD4 nadir, 103 vs. 164 (ns); body mass index, 20.8 vs. 23.7 (ns); diabetes mellitus, 27% vs. 4% (OR 8.8 [1.6–49] p = 0.01); median ALT, 39 vs. 26 IU/L (OR for above normal, 6.3 [1.2–34] p = 0.03); alk. phosphatase, 171 vs. 85 U/L (ns); platelets, 182 vs. 242x103/L (ns), current smoking, 7% vs. 41% (OR 0.11 [0.01–0.88] p = 0.04). Median cumulative exposure to ART (8.5 [IQR 5.1, 11.4] vs. 6.8 [3.3, 8.7] yrs, OR per yr exposure, 1.3 [1.0, 1.6] p = 0.02), NRTI (OR 1.3 [1.1, 1.7] p = 0.01), DDI (3.4 [1.5, 8.1] p = 0.005), ritonavir (1.4 [1.0, 1.9] p = 0.03) and nelfinavir (1.4 [1.0, 1.90] p = 0.03) were longer in cases. Exposure to NNRTI and PI were not different between groups. The association of NCPH with DDI exposure was robust in bivariable models incorporating the other covariables; low CD4 cell count was not a risk factor.
Conclusions: We found a strong association with prolonged exposure to DDI and the development of NCPH.

P162

Prolonged antiretroviral therapy (ART) and risk for chronic elevation of alanine aminotransferase (ALT) in HIV-infected persons without hepatitis B or C virus co-infections

Kovari H., Ledergerber B., Battegay M., Furrer H., Hirschel B., Cavassini M., Vernazza P., Bernasconi E., Mueller N., Weber R. and the Swiss HIV Cohort Study
University Hospital Zurich, Division of Infectious Diseases and Hospital epid. Zurich, Switzerland

Chronic liver disease in HIV-infected patients is mostly due to hepatitis virus co-infections or alcohol use. ART-related liver toxicity is recognized but rare.
Methods: We studied the incidence of and risk factors for new and chronic elevation of ALT in participants of the Swiss HIV Cohort Study, seen between 2002–2006, without HBV/HCV infections, with >3 semi-annual visits, and normal baseline ALT. Chronic ALT elevation was defined as ALT >50 (males)/>35 U/L (females) at >2 consecutive semi-annual visits. Poisson regression analysis was used.
Results: 2445 participants were followed for 9795 person-years (median age 39 yrs; 66.6% male; 83.7% Caucasian; HIV transmission 54% heterosexual, 40% homosexual, 1% IDU; median CD4 cells 436/microL; 31.5% ART naive; 57.9% on ART, 10.6% interrupted ART). 365 (14.9%) newly developed chronic ALT elevation (incidence 3.73 [95% CI 3.36–4.13] per 100 pyrs). Univariable analyses showed associations between chronic elevated ALT and increased BMI, increased waist circumference, fat accumulation, fat loss, hypertension, plasma cholesterol, triglyceride, and time-updated cumulative exposure to NRTI, NNRTI, and PI. Multivariable models, considering that many of these variables are collinear or on the same causal pathway, confirmed the association between chronic ALT elevation and increased body mass index (IRR per kg/m^2 1.06 [95% CI 1.03–1.09] p <0.001), ART per yr of exposure (IRR 1.04 [1.01–1.07] p = 0.004), NRTI per yr exposure (1.04 [1.02–1.08] p = 0.003), PI per yr exposure (1.04 [1.01–1.08] p = 0.013), but not with NNRTI exposure (IRR 1.03 [0.98–1.08]). Median alcohol use among non-abstinent patients was 10 g/d in persons with and without ALT elevation. Compared with no alcohol use (30% of patients), light (women <20 g/d, men <40 g/d; 57%) and moderate (20–40/40–60 g/d; 9%) alcohol use was not, but high use (>40/>60 g/d; 4% of patients) was associated with chronic ALT elevation (IRR 1.83 [1.16–2.87] p = 0.009). Treatment outcome and changes as well as mortality did not differ between patients with and without ALT elevation.
Conclusions: The incidence of chronic ALT elevation was 3.7/100 pyrs in patients without hepatitis virus co-infections. In multivariable models, prolonged antiretroviral therapy, NRTI exposure, PI exposure, increased BMI, and high alcohol use were associated with chronic ALT elevation. Long-term follow-up is needed to assess whether chronic ALT elevation will result in increased morbidity or mortality.

P163

Impact of HIV status on outcome of infectious complications in intravenous drug users

Mertz D., Viktorin N., Wolbers M., Flückiger U., Battegay M.
University Hospital Basel, Division of Infectious Diseases, Basel, Switzerland

Objectives: Comparison of HIV positive to HIV negative intravenous drug users (IDU) in management and outcome of infections.
Methods: Retrospective analysis of all infections in IDU from 1/2001–12/2006 seen by the infectious diseases service in the University Hospital of Basel.
Results: We analysed 344 episodes (224 HIV negative, 120 HIV positive). The most frequent infections were skin and soft tissue infections in both groups. During episodes, non-compliance (e.g. non-adherence to treatment during hospitalization) was 21.9% and 15.8% for HIV negative and positive status, respectively. This difference was not significant, while psychiatric disorders had a significant negative impact on compliance overall (OR 2.38, CI 1.11–5.12, p = 0.026). Readmission rate within 30 days was 13.7%; 3.8% due to relapse. The overall readmission rate was slightly higher for HIV infected patients (60.8%) than others (51.3%) but not significantly so (p = 0.13). Survival status was available in 92.1% (median follow-up 35.6 months): Infection-related deaths (OR 1.94, CI 0.77-4.88, p = 0.161), overall in hospital mortality (OR 2.38, CI 1.01–5.60, p = 0.047) and mortality during the entire follow up period (OR 2.36, CI 1.27–4.37, p = 0.006) all tended to be increased in HIV positive episodes.
Conclusions: In conclusion, we have not found a significant impact of HIV status on compliance during hospitalization and the frequency of relapses. However, HIV positive IDUs show a significantly higher mortality during hospitalization and in the follow up.

P164

Pharmacotherapy on the edge: HIV salvage therapy in a patient after renal transplantation

Mertz D., Battegay M., Marzolini C., Mayr M.
University Hospital Basel, Division of Infectious Diseases, Basel, Switzerland

Concomitant use of immunosuppressive agents and antiretroviral drugs may lead to complex drug-drug interactions. The calcineurin inhibitor (CNI) tacrolimus is metabolized by cytochrome P450 3A4 and is a substrate of P-glycoprotein. Both pathways can be inhibited by protease inhibitors (PI) and therefore significantly increase tacrolimus trough levels. In a patient with HIV-associated focal segmental glomerulosclerosis leading to renal cadaveric transplantation, HIV salvage therapy was started with the new PI darunavir, boosted with ritonavir, another PI. The reduction of the first-pass and post-absorptive metabolism of tacrolimus by PI led to a dramatic increase in tacrolimus trough levels and extreme prolongation of the half-life. Finally, stable tacrolimus trough levels were maintained by a single dose of 0.5 mg per week corresponding to 3.5% of the basic dose. Our case highlights that the co-administration of PI and tacrolimus is feasible through intense reduction in dose and prolongation of the dosing interval of the CNI. Complex drug interactions may become more frequent due to more HIV-infected patients being transplanted and newer HIV drugs being used. Close monitoring and excellent adherence are mandatory to avoid the risk of harm for the graft and the patient.

P165

Efficient suppression of minority quasispecies of drug-resistant viruses present at primary HIV-1 infection by boosted protease inhibitor containing antiretroviral therapy

Metzner K., Rauch P., von Wyl V., Kuster H., Böni J., Weber R., Günthard H.
University Hospital Zurich, Division of Infectious Diseases + Hospital Epid., Zurich, Switzerland

It has been shown that a substantial fraction of acutely infected patients harbor minority quasispecies of drug-resistant viruses as measured by the very sensitive method of allele-specific real-time PCR (AS-PCR). We have previously demonstrated that rapid selection of those minor populations can lead to virological failure in patients receiving ART regimens with a low genetic barrier. Here, we studied the fate of those minor variants during the first weeks of ART containing a boosted PI, thus, a regimen with a high genetic barrier. Baseline plasma samples from 93 acutely infected patients from the Zurich Primary HIV-1 Infection study were collected between March 2002 and April 2007 and tested by AS-PCR for the K103N and M184V variants (discriminative power: 0.01 and 0.2%, respectively). The K103N mutation was detected in 6/93 patients (6.5%) and the M184V in 11/91 patients (12.1%) at baseline. These variants represented 0.08 to 3.76% and 0.4 to 8.3% of plasma viral genomes in a total of 15/93 patients (16.1%), respectively. Using conventional population sequencing, none of these mutations were detected in any patient. Follow-up samples of 5 patients harboring minority quasispecies (4 pts: M184V, 1 pt: K103N) of drug-resistant viruses at baseline and 5 control patients were measured by AS-PC during the first 12 wks of ART. The ART regimen contained LPV/r+AZT,+3TC (1 pt: LPV/r+TDF+ddI). All patients showed a rapid decline in viral load and neither K103N nor M184V were detected. 4 patients stopped suppressive ART after 42–87 wks, the other 6 patients show no evidence of treatment failure within the last 80–275 wks. Minority quasispecies, in particular viruses harboring the M184V mutation at baseline were efficiently suppressed in acutely infected patients receiving a boosted PI and two NRTIs including 3TC in the majority. Under this high genetic barrier regimen, the M184V was not further selected.

The numbers refer to the pages of this supplement.