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Implementing an educational smoking cessation curriculum for medical students: a randomised, controlled trial

D. Stolz[1], W. Langewitz[1], A. Meyer[1], K. Pierer[1], P. Tschudi[1], W. Strobel[1], A. Perruchoud[1], K. Fagerstroem[2], M. Tamm[1].
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Background: It is crucial that medical students – the future medical practitioners – have adequate training and skills in treating tobacco dependence by promoting smoking cessation. However, only 27% of last-year European medical students believe to have sufficient knowledge to counsel smokers. This study aimed to implement and evaluate the impact of enhanced methods of tobacco-intervention training during medical school at the University of Basel.
Methods: After having accessed a web-based learning platform about smoking cessation, 140 third-year medical students were randomly exposed to one of 4 different tobacco education approaches, two hours each: 1) idendependent learning using web-based material; 2) intervention tutorial (lecture); 3) learning patient-centered counseling through role playing in small groups; 4) interaction with real patients under supervision. The primary end-points were the scores of objective structured clinical examination (OSCE) as well as self- and external-assessment of counseling-skills and MCQ for testing theoretical knowledge.
Results: OSCE results differed significantly between the four educational approaches (p = 0.007). Scores were highest in group 4 (35.9 ± 8.7), followed by group 3 (35.7 ± 6.5), group 2 (33.5 ± 9.4) and group 1 (28.0 ± 9.6). Similarly, the percentage of students showing fully sufficient counseling skills was highest in group 4 (60.7%) and 3 (57.7%), followed by group 2 (34.8%) and group 1 (30%) (p = 0.043). Although all groups showed improvement in smoking cessation awareness during the course (p <0.0001), self-assessment of cessation-skills showed a significantly superiority in groups 3 and 4 as compared to groups 1 and 2 (p <0.0001). The percentage of students feeling "completely sure" or "sure" about their counseling skills was 76.9% in group 3, 62.5% in group 4, 40.7% in group 2 and 19.5% in group 1 (p <0.0001). Student-satisfaction was most in groups 3 (8 [7–9]) and 4 (8 [7–9]) and significantly less in groups 1 (5 [3–7]) and 2 (7 [5–8]) (p = 0.006). There was no difference in the scores reflecting theoretical knowledge (p = 0.439).
Conclusion: Neither a web-based learning platform nor a lecture are sufficient for providing counseling skills required for smoking cessation. In contrast, role play in small groups and interaction with real patients under supervision are equally efficient in increasing counseling skills and self-confidence. All didactic approaches studied are similar in providing theoretical knowledge.

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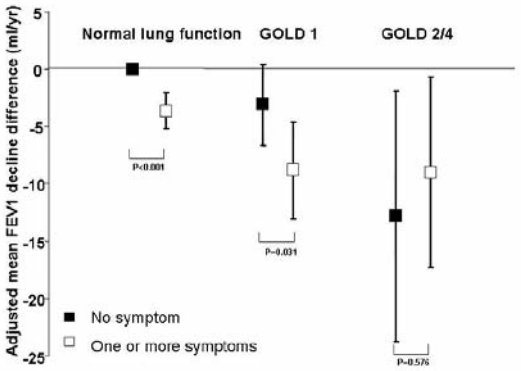
GOLD stage 1 COPD: long-term lung function decline, utilisation of care and quality of life

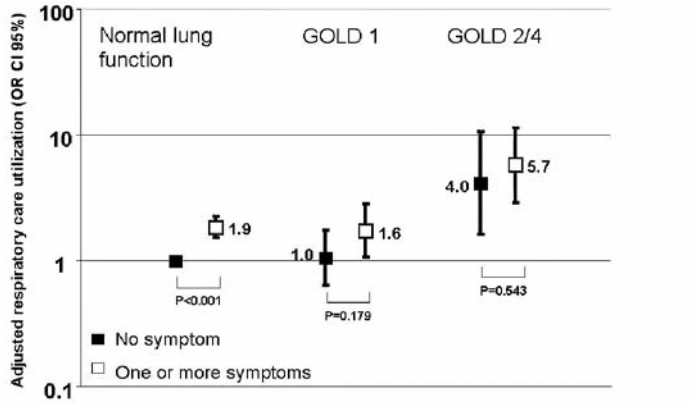
P-O. Bridevaux, M.W. Gerbase, N.M. Probst-Hensch, C. Schindler, J.-M. Gaspoz, T. Rochat for the SAPALDIA team

Background: Little is known on the long term outcomes of individuals with mild COPD, as defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD).
Methods: A population cohort of 6671 randomly selected adults without asthma was stratified into categories of GOLD-defined COPD. Further stratification was based on the presence or absence of respiratory symptoms. After 11 years, associations between baseline categories of COPD and FEV_1 decline, respiratory care utilization, and quality of life as measured by the SF-36 questionnaire, were examined after controlling for age, sex, smoking and educational status.
Results: At baseline, GOLD criteria were met by 610 (9.1%) participants from whom 519 (85.1%) had GOLD 1 COPD. At follow-up, individuals with symptomatic GOLD 1 COPD (n = 224) had faster FEV1 decline (–9 ml/yr [CI 95% –13; –5]), increased respiratory care utilization (OR 1.6 [CI 95% 1.0; 2.6]) and lower quality of life compared to asymptomatic subjects with normal lung function (n = 3627, reference group). By contrast, asymptomatic GOLD 1 COPD subjects (n = 295) had no differences in FEV_1 decline (–3 ml/yr [CI95% –7; +1]), respiratory care utilization (OR 1.0 [CI 95% 0.7; 1.7]) or quality of life scores when compared to the reference group.
Conclusions: In population-based studies, respiratory symptoms are of major importance for predicting long-term clinical outcomes in COPD subjects with mild obstruction. Population studies that are based on spirometry only may misestimate the prevalence of clinically relevant COPD.

Adjusted FEV_1 decline difference* (ml/yr with 95% confidence interval) over 11 years, stratified by GOLD and symptom† categories at SAPALDIA 1 (1991)



Adjusted odds ratio of respiratory care utilization (with 95% confidence interval) at SAPALDIA 2 (2002), stratified by GOLD and symptom† categories at SAPALDIA 1 (1991)



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Sex-specific effect of weight change on systemic inflammation in subjects with accelerated lung function decline. A population study

P-O. Bridevaux, C. Schindler, M.W. Gerbase, N.M. Probst-Hensch, U. Ackermann-Liebrich, J-M. Gaspoz, T. Rochat for the SAPALDIA team

Rationale: Chronic obstructive pulmonary disease (COPD) is an independent risk factor for cardiovascular disease due, in part, to higher level of systemic inflammation. Obesity, another condition associated with systemic inflammation may interact with COPD and could be a target to reduce systemic inflammation in subjects with early COPD.
Methods: We measured systemic inflammation (high-sensitivity C-Reactive Protein [hs-CRP]), weight gain and FEV1 decline in 5666 randomly selected subjects from the SAPALDIA cohort (Swiss Study on Air Pollution and Respiratory Disease in Adults), followed for 11 years. Of those, 1417 had COPD defined by accelerated FEV_1 decline (mean FEV_1 decline 73 ml/year [SD 22]). Mixed linear models were applied to estimate differences of hs-CRP between subjects in each weight change categories. Hs-CRP levels were expressed in geometric mean.
Results: After adjustment for age, smoking, baseline FEV_1, socio-economical status, physical activity and study area, weight gain was associated with significantly higher level of hs-CRP. (see figure) In women with COPD, the effect of weight gain on hs-CRP was larger than in men.
Conclusions: In women with fast FEV_1 decline, systemic inflammation related to weight gain is higher than in men. Higher systemic inflammation associated with sex-specific weight gain may in part explain the worse prognosis of COPD in women. How weight control in women is amenable to modify the level of systemic inflammation and the prognosis of COPD is unknown.

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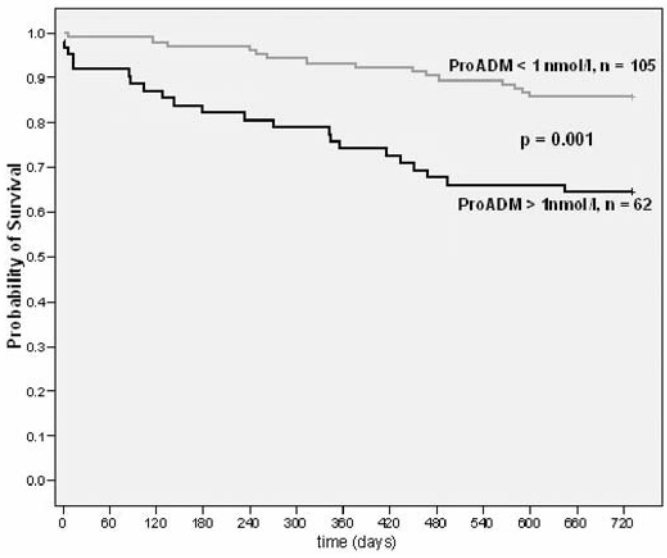
Plasma pro-adrenomedullin but not plasma pro-endothelin predicts survival in exacerbations of COPD

D. Stolz[1], M. Christ-Crain[1], N. Morgenthaler[2], D. Miedinger[1], J. Leuppi[1], C. Müller[1], R. Bingisser[1], J. Struck[2], B. Müller[1], M. Tamm[1]. 1 University Hospital Basel (Basel, CH); 2 Brahms AG (Hennigsdorf, D)

Background: Plasma endothelin and adrenomedullin are increased in pulmonary arterial hypertension, hypoxia and pulmonary infections, conditions that predict survival in patients with COPD. We investigated whether plasma pro-endothelin-1 and/or pro-adrenomedullin at admission to hospital for acute exacerbation predict survival in patients with COPD.
Methods: We examined 167 patients admitted to hospital for acute exacerbation and we followed them up for 2 years. We measured plasma C-terminal pro-endothelin-1 and mid-regional pro-adrenomedullin at admission, after 14–18 days, and at 6 months. In addition to plasma C-terminal (CT) pro-endothelin-1 and mid-regional (MR) pro-adrenomedullin we assessed with Cox-regression univariate and multivariate analyses the predictive value of clinical, functional, and laboratory parameters on 2 year survival. We analyzed time to death by Kaplan-Meier curves.
Results: As compared to recovery and stable state, CT-pro-endothelin-1 and MR-pro-adrenomedullin were significantly increased at admission ($p < 0.0001$ and $p = 0.002$, respectively). MR-pro-adrenomedullin but not CT-pro-endothelin-1 was associated with increased in-hospital mortality ($p = 0.049$), and independently predicted 2 year survival ($p = 0.002$). ProADM plasma levels >1.0 nmol/l on admission increased the mortality risk within two years from 14% to 36% (Figure, $p = 0.002$). By contrast, age ($p = 0.893$), comorbidities ($p = 0.267$), FEV_1% predicted ($p = 0.882$), PaO_2 ($p = 0.427$), $PaCO_2$ ($p = 0.616$), leukocyte counts ($p = 0.642$), C-reactive protein ($p = 0.789$), procalcitonin ($p = 0.104$), pulmonary arterial hypertension ($p = 0.366$), and CT-pro-endothelin-1 ($p = 0.311$) were not independently associated with 2 year survival.
Conclusions: This study shows that plasma pro-adrenomedullin but not plasma pro-endothelin-1 at admission to hospital for an acute exacerbation independently predicts survival, thus suggesting that this biomarker could be used to predict prognosis in patients with COPD.



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Prevalence and geographic distribution of sarcoidosis in Switzerland

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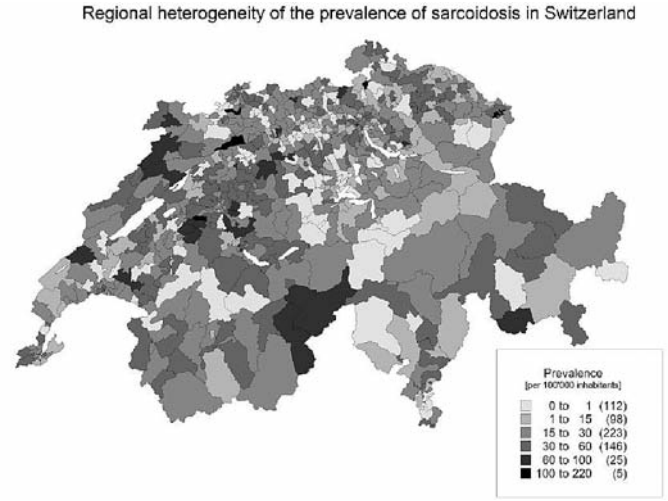
Introduction: The reported prevalence of sarcoidosis varies considerably in different countries and studies. Typically, it is estimated at 1–40 per 100'000 inhabitants. The aim of the current study was to investigate the prevalence and regional distribution of sarcoidosis in Switzerland. Furthermore, we studied correlations between the regional prevalence of sarcoidosis and measures of air quality, regional importance of agriculture, metal or other industries.
Methods: Cases with sarcoidosis were identified from the hospital statistics (Federal Office for Statistics, Years 2002–2005). The prevalence resulting from this in-patient data pool was adjusted with the aid of the incidence of newly diagnosed sarcoidosis from biopsy results in the City of Basel (in- and out-patients; Pathology, University Hospital Basel). Regional exposure characteristics included air quality measurements (MeteoSwiss, PM10, Year 2002), and regional distribution of different industrial sectors focusing on agriculture and metal industry (Federal Office for Statistics, NOGA, Year 2005). The geographic resolution is defined by postal codes (Swiss Post). For the probability distribution generalized linear models with the Poisson's distribution were used. Each exposure co-variable was individually tested for significance to the number of sarcoidosis cases per region. All significant co-variables were then collectively scrutinized for interaction and interdependence with a generalized linear model with a backward optimization strategy.
Results: We found a mean incidence of sarcoidosis of 7 [CI 95% 5 to 11] and a mean prevalence of 98 [CI 95% 72 to 123] per 100'000 inhabitants. We observed a significant regional heterogeneity, which was not explained by regional differences in the medical services nor the density of physicians. The presence of different types of metal industry and the proportion of grassland agriculture were positively associated with the regional prevalence of sarcoidosis. Air quality did not correlate with the number of sarcoidosis cases per region.
Conclusions: The prevalence of sarcoidosis in Switzerland was approximately 5–10 times higher than assumed based on estimates from specialized institutions. There were significant regional differences in Switzerland. We found a higher prevalence in regions with metal industry, and a high agricultural production, especially grassland farming.



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Stem cells derived from rat bone marrow accelerate alveolar epithelial repair in vitro and reduce bleomycin-induced pulmonary fibrosis in a lung tissue culture model

A. Gazdhar[1], F. Blank[2], P. Gehr[2], T. Geiser[1].
1 University Hospital Berne (Berne, CH);
2 Institute of Anatomy (Berne, CH)

Abnormal alveolar epithelial wound repair after injury results in the development of pulmonary fibrosis. Improving alveolar epithelial repair may therefore result in a reduction of pulmonary fibrosis. We hypothesized that bone marrow derived mesenchymal stem cells (BMSC) in co-culture with alveolar epithelial cells may accelerate alveolar epithelial wound repair in vitro and reduce bleomycin induced pulmonary fibrosis in a lung tissue slice culture model. A549 alveolar epithelial cells were grown to a monolayer on top of a porous membrane at an air-liquid interface and rat BMSC were grown attached on the opposite side of the membrane. After wounding of the confluent alveolar epithelial cell monolayer, the rate of wound repair was determined. In addition, 200 µm lung tissue slices from normal and bleomycin injured rat lungs were cultured in presence and absence of 1x106 rat BMSC. At 24 hrs after wounding, alveolar epithelial wound closure in vitro was increased in presence of BMSC compared to medium control (51% wound closure at 24 hours compared to complete healing with BMSC, $p < 0.05$). Confocal microscopy showed marked cellular interaction between BMSC and alveolar epithelial cells at the wound edge, but not in areas of the intact epithelial cell layer. At day 5 the hydroxyproline content of the lung slice treated with BMSC was reduced (1.5 ± 0.29 µg/mg) as compared to control (6.05 ± 3.1 µg/mg). In presence of BMSC, alveolar epithelial wound repair in vitro was increased and bleomycin induced fibrosis was reduced in the lung tissue culture model. Further studies will elucidate possible mechanisms of the beneficial effect of BMSC on alveolar epithelial repair and fibrosis.

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Cell-specific gene transfer of hepatocyte growth factor to alveolar type II epithelial cells reduces bleomycin-induced pulmonary fibrosis

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1 University Hospital Berne (Berne, CH); 2 Departement of Clinical Research (Berne, CH); 3 Pathology (Berne, CH)

Abnormal alveolar wound repair contributes to the development of pulmonary fibrosis after lung injury. Hepatocyte growth factor (HGF) is a potent mitogenic factor for alveolar epithelial cells and improves alveolar epithelial repair in vitro and in vivo. We have recently shown that electroporation-mediated gene transfer of hHGF decreases bleomycin-induced pulmonary fibrosis, mainly by improving alveolar epithelial repair (Am J Physiol Lung 2007). We further developed our HGF gene transfer method by targeting specifically the alveolar type II epithelial cells in vivo in order to increase efficiency and reduce possible side effects of HGF gene transfer to the whole lung. A plasmid encoding human HGF, [pSpChHGF = human HGF gene expressed from the human Surfactant protein C promoter] was designed and extracorporal electroporation-mediated, nonviral gene transfer of HGF in vivo was performed 7 days after bleomycin-induced lung injury in the rat.
Extracorporal electroporation-mediated in vivo HGF gene transfer using pSpChHGF reduced pulmonary fibrosis as assessed by histology and hydroxyproline determination 14 days after bleomycin instillation compared with controls treated with the same vector not containing the HGF sequence (pSpC). Co-staining experiments revealed that hHGF was specifically expressed in alveolar type II epithelial cells. In conclusion, extracorporal electroporation-mediated gene transfer of hHGF specifically to the alveolar type II epithelial cells is feasible and decreases bleomycin-induced pulmonary fibrosis. Cell-specific HGF gene transfer to alveolar type II epithelial cells may therefore represent a novel therapeutic approach in patients with pulmonary fibrosis.

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Exploration of the transcription factor activity of the glucocorticoid mometasone using RLQ analysis of gene expression microarray data

F. Baty, M. Facompré, J. Rüdiger, M. Brutsche.
Universitätsspital Basel (Basel, CH).

Background: Complex regulatory networks involving transcription factors generally orchestrate the regulation of gene expression. There are novel statistical tools that allow to combine external information on both genes (e.g. transcription factor binding sites (TFBS) annotations) and samples (experimental design) in the analysis of gene expression microarrays.
Methods: We used RLQ analysis (Dolédec et al., Environ. Ecol. Stat., 1996) which aims to study the joint structure of three tables: a central table L (gene expression data), an experimental design table R (sample annotations) and a table Q with variable informations (gene annotations). The approach was applied to a transcriptomics data set investigating the mechanisms of action of the glucocorticoid mometasone in the proliferation of fibroblasts. Gene profiles of two cell lines were monitored at 8 different time points using Affymetrix GeneChips hgu133a. Enriched TFBS were extracted using the oPOSSUM web tool and RLQ was performed using the R library ade4.
Results: Using RLQ, it was possible to uncover potential transcription factor-mediated gene regulation of mometasone (Figure 1). Genes which were early down-regulated (1.5–2 hours) presented an enrichment of NF-kappaB, RelA and FOS TFBS. Genes with a late up-regulation (6 hours) were clearly enriched for Spi-B TFBS.
Conclusion: For the first time, we successfully applied RLQ to associate promoter motifs with gene dysregulations in a time course experiment of microarray data. The flexibility of RLQ allows to include a large variety of additional gene annotations. This greatly facilitates the systematic interpretation of gene expression data sets. The transcription factor Spi-B was recently described as being involved in the steroid auto-regulation of the human glucocorticoid receptor (hGR). The analysis of Spi-B / mometasone interactions may represent an important axis of research for the understanding of steroid mechanisms of action.

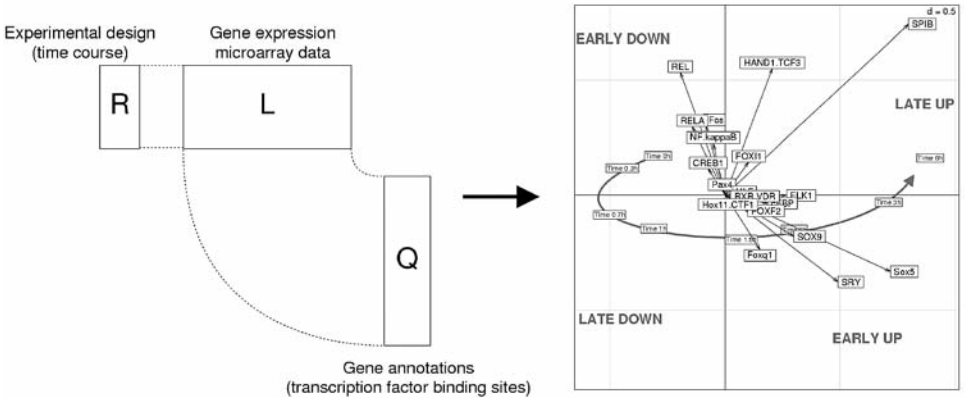

Figure 1: RLQ analysis of the importance of transcription factor binding sites for the mechanism of action of mometasone in proliferating primary lung fibroblasts in vitro.

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Lymph node stromal cells promote naïve T-cell survival via an IL-7-dependent mechanism

T.K. Vogt[1], A. Link[1], S. Favre[1], M.R. Britschgi[1], H. Acha-Orbea[1], B. Hinz[2], J.G. Cyster[3], S.A. Luther[1].
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Naïve T-cells require continual signals from their microenvironment to maintain viability. In vivo, naïve T-cells survive for several weeks. However, once transferred in vitro they die within one to two days. Interleukin-7 (IL-7) was shown to be the most critical cytokine for the survival of naïve T-cells both in vivo and in vitro. At present, the cells that produce IL-7 and support T-cell survival in the periphery are poorly defined. Several lines of evidence point to an important role for secondary lymphoid tissues in naïve T-cell survival.
As dendritic cells are a poor source of IL-7, we have focused on the poorly characterized gp38+ T-zone stromal cells. To test whether T-zone stromal cells are able to promote naïve T-cell survival, we established an in vitro co-culture system of lymphocytes on top of a layer of gp38+ lymph node stromal cells. Indeed, an enriched population of gp38+ stromal cells was able to promote CD4+ and CD8+ T-cell survival in vitro. Blocking of IL-7 signaling strongly reduced the survival promoting effect of the stromal cells. In agreement with this observation, no direct cell-cell contact was required between stromal cells and T-cells. In contrast, gp38+ stromal cells had no effect on the survival of naïve B cells consistent with their lack of IL7-receptor expression. In summary, we provide strong evidence for T-zone stromal cells being an important source of IL-7 within secondary lymphoid organs, which is likely to be critical for naïve T-cell homeostasis.

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IL-21R signalling is essential for the maintenance of CD8 T-cell responses in persistent LCMV docile infection

A. Fröhlich, J. Kisielow, J. Weber, A. Oxenius, M. Kopf.
ETH Zürich (Schlieren, CH)

Interleukin 21 (IL-21) is the latest member of the common gamma chain gc family of cytokines including IL-2, IL-4, IL-7, IL-9 and IL-15, which influence a broad spectrum of immunological responses. We generated IL-21R-deficient mice and found that IL-21 is a key cytokine for development of Th2 allergic airway responses and Th2 type inflammatory responses induced by nematodes. In contrast, Th1 responses to L. major and Th17 mediated autoimmune myocarditis was unaffected in IL-21R-/- mice.
Some studies have suggested that IL-21 can enhance CD8 T cell responses in vitro. Therefore, we have investigated the contribution of IL-21R signaling in immune responses to infection with lymphocytic choriomenigitis virus (LCMV), influenza virus, and vaccinia virus. IL-21R-/- and B6 wildtype mice showed comparable CTL and CD4 T cell responses at day 7 after infection with low dose LCMV Docile (200 pfu). However, the number of virus specific CD8 T cells was remarkably reduced in IL-21R-/- mice during the contraction phase of the response starting at day 11. CD8 T cell exhaustion observed in wildtype mice upon infection with high dose (10^6pfu) LCMV Docile was much more pronounced and associated with high virus titers in IL-21R-/- mice. Similarly, a preferential loss of IL-21R-/- CD8 T cell number and activity in response to LCMV Docile was observed >15 day after infection of mixed chimeras generated by transfer of bone marrow from IL-21R-/- CD45.2 mice and IL-21R+/+ CD45.1 mice into lethally irradiated C57BL/6 CD45.2 recipients.
Interestingly, CD8 T cell responses were not imparired in IL-21R-/- mice infected with non-persistent cytotoxic viruses such as influenza virus and vaccina virus.
Thus, our results suggests that IL-21 promotes control of infection with persistent viruses by by maintaining CD8 T cell function and protection from exhaustion.

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Expansion of the peripheral Treg compartment by FLT3L treatment

L. Swee, A. Rolink. University of Basel (Basel, CH)

Daily injections of 5–10 µg recombinant FLT3L for 7–10 days resulted in a 2-fold increase in the number of regulatory T cells (Treg). A similar expansion of Treg was found in thymectomized mice injected with FLT3L, indicating that this increase was thymus independent. Moreover, in FLT3L- treated mice the percentage of Tregs expressing the cell cycle antigen Ki67 was increased by a factor of 2. On the other hand, no increase in the percentage of Ki67 expressing cells was observed amongst conventional T cells in these treated mice. Therefore, the expansion of Tregs by FLT3L seems to be due to proliferation rather than conversion of conventional T cells into Treg. Tregs derived from FLT3L-treated mice were in in vitro assays, as active as their counterparts from untreated mice. FLT3L-treated mice showed a normal T cell-dependent humoral immune response and rejected allogeneic skin grafts with the same kinetics as controls. Thus, the increase of Tregs did not result in a generalized immunodeficiency status. However, FLT3L treatment protected (C57BL/6 x DBA/2)F1 mice from an acute lethal graft versus host disease induced by the injection of C57BL/6 T cells. Even more surprising, FLT3L treatment at one week after the induction of the graft versus host reaction inhibited the development of this lethal disease. Currently the role of Tregs in the prophylaxis and therapy of acute graft versus host disease is under investigation.

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Synergy-inducing chemokines enhance CCR2 ligand activity on monocytes

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Introduction: The migration of monocytes at site of inflammation is largely determined by their response to chemokines. While the chemokine specificities and expression patterns of chemokine receptors are well defined, it is still a matter of debate how monocytes integrate the messages provided by different chemokines that are concomitantly produced in physiological or pathological situations in vivo.
Results: We present evidence for a regulatory mechanism of monocyte trafficking. In the presence of non-agonist, unrelated chemokines constitutively expressed in secondary lymphoid organs (CCL19 and CCL21), in vitro migration of human monocytes towards the CCR2 ligand CCL7 could occur at much lower concentration. MAPK activation and enzyme release induced by various CCR2 agonists were synergistically augmented when non-ligand chemokines were present. The synergistic effect is mediated by CCR2 as it can be reproduced in a mouse cell line expressing only the human CCR2. As many chemokines are known to interact with glycosaminoglycans (GAGs), we shed GAGs from the cell surface before chemotaxis to exclude their involvement in mediating synergism. This treatment did not affect chemotaxis induced by CCL7 alone, nor the synergistic increase in the presence of CCL19 and CCL21. Binding studies on CCR2+ cells showed that synergy-inducing chemokines do not compete with the CCR2 agonist CCL2. In order to investigate whether the presence of CCL19 and CCL21 can influence the internalization of the CCR2 agonists, we studied the potential of CCR2 or of the decoy receptor D6 to internalize CCL2 and CCL7 in the presence of the synergy-inducing chemokines. Their presence can prevent the internalization of CCL7 by either receptor. The effect of long term exposure of monocytes to synergy-inducing chemokines is under investigation by gene chip array.
Conclusion: Chemokine-induced synergism is a general phenomenon involving several chemokine receptor and might provide an amplification system in “chemokine-rich” tissues, rendering monocytes more competent to respond to migratory cues.

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Human inflammatory TH17 cells: phenotype and differentiation requirements

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Interleukin 17 (IL-17)-producing T helper cells (TH17 cells) have been characterized in mice as a distinct subset of effector cells, but their identity and properties in humans remain elusive. We report here that expression of CCR6 and CCR4 together identified human memory CD4+ T cells selectively producing IL-17 and expressing mRNA encoding the human ortholog of mouse RORgammat, a transcription factor, whereas CCR6 and CXCR3 identified TH1 cells producing interferon-gamma and T helper cells producing both IFN-gamma and IL-17. Memory T cells specific for /Candida albicans/ were present mainly in the CCR6+CCR4+ TH17 subset, whereas memory T cells specific for Mycobacterium tuberculosis were present in CCR6+CXCR3+ T helper type 1 subset. The elicitation of IL-17 responses correlated with the capacity of /C. albicans/ hyphae to

stimulate antigen-presenting cells for the priming of TH17 responses in vitro and for the production of IL-23 but not IL-12. In addition we found that prostaglandin E2, a proinflammatory mediator abundant in chronically inflamed tissues, beside its well known effect on the modulation of the IL-23/IL-12 balance by antigen presenting cells, can also skew the IL-17/IFN-gamma balance by directly acting on the EP2 and EP4 receptors expressed on CD4 T cells. Indeed PGE2 was able to increase IL-17 and inhibit IFN-gamma release by purified memory cells and it preferentially inhibited the proliferation of the CCR6- non IL-17 producing memory subsets. Our results demonstrate that human TH17 cells have distinct migratory capacity and antigenic specificities and provide a rationale for the beneficial effect of COX-2 inhibitors in the treatment of chronic inflammation.

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Relationship between degranulation activity and content of granules in virus-specific CD8 T-cells

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Background: Degranulation activity, as measured by CD107a mobilization, is commonly used to define cytotoxic CD8 T-cells. However, the relationship between granule's content and cytotoxic capacity of virus-specific CD8 T-cells was never investigated.
Methods: A variety of virus-specific CD8 T-cell responses including CMV (n = 16), EBV (n = 21) and Flu (n = 17) were identified using tetramer complexes or peptide stimulation and analyzed for CD107a mobilization, as well as for the simultaneous expression of perforin (Perf), granzyme (Grz) A, GrzB and GrzK by polychromatic flow cytometry combined with CD127 (i.e. IL-7Ra), CCR7 and CD45RA, that were used to assess T-cell differentiation.
Results: Polychromatic flow cytometric analyses combining GrzA, GrzB, GrzK and Perf on CD8 T-cells identified 16 distinct populations. Highly differentiated cells (CCR7-CD127-CD45RA±) mostly contained Perf and GrzB, but not GrzA or GrzK, which in contrast were mostly found in poorly differentiated CD8 T-cells (CCR7+CD127+CD45RA-). Interestingly, analysis of the different viruses showed typical virus-specific patterns. CMV-specific T-cells were mostly composed of four distinct subsets and >35% of cell were Perf+GrzB+GrzA+GrzK±. Three distinct subsets were observed in EBV-specific T-cells and >40% of cell were Perf-GrzK+GrzB±GrzA±. Whereas above 60% of Flu-specific T-cells were Perf-GrzK+GrzB-GrzA-. Differences between the viruses were highly significant (All P <0.01). Of note, Flu-specific CD8 T cells, i.e. lacking perf and GrzB, did not show direct ex vivo cytotoxicity despite the fact that a similar level of degranulation activity was observed in Flu-, EBV- and CMV-specific CD8 T cells (ranging from 40–60%, P >0.05) following stimulation with the same peptides.
Conclusions: The composition of granules is highly heterogeneous in CD8 T-cells and associated with differentiation. However, typical patterns of granule's content are observed for the different viruses and lack of perforin is associated with the absence of cytotoxic activity. In contrast, degranulation activity is consistently observed in all models of virus infection (including non cytotoxic responses) and therefore represents an irrelevant marker of CD8 T-cells with cytotoxic capacity.

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APRIL is critical for plasmablast survival in the bone marrow and poorly expressed by early life bone marrow stromal cells

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The persistence of serum IgG antibodies elicited in human infants is much shorter than when such responses are elicited later in life. The reasons for this rapid waning of antigen-specific antibodies elicited in infancy are yet unknown. We have recently shown that adoptively transferred tetanus toxoid (TT)-specific plasmablasts (PBs) efficiently reach the bone marrow (BM) of infant mice. However, TT-specific PBs fail to persist in the early life BM, suggesting that they fail to receive the molecular signals that support their survival/differentiation. Using APRIL and BAFF/BLyS deficient mice, we demonstrate here that APRIL is a critical factor for the establishment of the adult BM reservoir of anti-TT IgG secreting cells. Through in vitro analyses of PB/PC survival/differentiation, we show that APRIL induces the expression of Bcl-XL by a preferential binding to heparan sulfate proteoglycans at the surface of CD138+ cells. Last, we identify BM resident macrophages as the main cells that provide survival signals to PBs and show that this function is slowly acquired in early life, in parallel to a progressive acquisition of APRIL expression. Altogether, this identifies APRIL as a critical signal for PB survival that is poorly expressed in the early life BM compartment.

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Distinct mechanisms regulate mucosal and systemic IgA responses against virus-like particles

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IgA represents the most prominent antibody (Ab) class at mucosal surfaces and its neutralizing properties can prevent infection of invading pathogens. In serum, IgA corresponds to a relatively small proportion of total serum Ab, but nevertheless it is important for elimination of pathogens which penetrated the mucosal barrier. For these reasons the induction of IgA is an important goal in vaccination. Induction of IgA responses can be induced in a T cell dependent (TD) as well as a T cell independent (TI) process. In general, the latter is more commonly related to intestinal IgA induced by the presence of commensal microbes.
Virus-like particles (VLPs) are antigen carriers capable to induce strong Ab responses due to their highly ordered structure. In the present study, we evaluate the mechanisms involved in the induction of mucosal and systemic IgA responses against VLPs.
For this purpose, C57BL/6, CD40-/- and TbetaRII-B (mice lacking expression of TGF-beta RII exclusively on B cells) were immunized either intranasally (i.n.) or subcutaneously (s.c.) with VLPs and 20 days later the IgA levels were measured by ELISA in bronchus-alveolar lavage (BAL) and serum.
The specific IgA levels in BAL of CD40-/- and TbetaRII-B mice immunized i.n. were strongly reduced when compared to the C57BL/6 group, suggesting that the mucosal IgA response induced in the airways is largely dependent of Th cells. However, surprisingly, the IgA levels in serum of CD40-/- mice immunized s.c. were similar or even higher when compared to the C57BL/6 control group at early time points. Additionally, the specific systemic IgA levels detected in the TbetaRII-B mice showed slight and not significant reduction compared to the control group. This indicates that the systemic IgA response generated after s.c. administration of VLPs is, at least partially, mediated by a TI mechanism.
Taken together, these results suggest that distinct mechanisms regulate mucosal and systemic IgA responses against VLPs. Furthermore it shows that the mechanisms strongly rely on the route of immunization, the target cells involved as well as the site where the Ab response is induced.

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CD40L+ CD4+ effector memory T-cells migrating into lymph nodes license dendritic cells for induction of autoimmunity

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Introduction: Triggering of CD40 by CD40L expressed on antigen-activated CD4+ T helper cells or by agonistic antibodies is sufficient to license dendritic cells (DCs) for priming of T cells against tissue antigens or antigens administered in the absence of adjuvant.
Results: We found that mouse effector memory CD4+ T cells (CD4+ TEM), but not naïve or central memory T cells (CD4+ TCM), constitutively expressed CD40L at levels sufficient to induce DC maturation in vitro and in vivo in the absence of antigenic stimulation. CD4+ TEM migrated in a CD62P-dependent fashion into lymph nodes that were induced to chronically express CD62P on high endothelial venules. Presentation of a MOG peptide by TEM-licensed DCs was sufficient to induce development of experimental allergic encephalomyelitis (EAE). DC maturation and autoimmunity were inhibited by antibodies to CD62P that prevented CD4+ TEM lymph node migration into lymph nodes.
Conclusion: These results provide a mechanistic link between lymphocyte traffic in lymph nodes and induction of autoimmune diseases.

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Both blood-derived and CNS-derived APC contribute to antigen presentation during the effector phase of EAE

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During experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis, MHCII expression is upregulated on a variety of different types of antigen presenting cells (APC) in the CNS. Defining the respective roles of antigen presentation by these APC during EAE remains a major challenge. To address this question we have generated mice exhibiting altered patterns of MHCII expression by exploiting the fact that the class II transactivator (CIITA) gene, which encodes the master regulatory factor of MHCII genes, is controlled by three cell-type specific promoters called pI, pIII and pIV. We recently generated a strain of mice lacking pI driven CIITA expression. The analysis of MHCII expression in pI-/- mice demonstrated that pI is essential for driving CIITA and MHCII expression in interferon-gamma activated macrophages and microglia. It is also required to a variable degree in various dendritic cell (DC) subsets. In contrast, MHCII expression is affected only marginally in thymic epithelial cells and B cells. pI is thus critical for driving CIITA and MHCII expression in the APC (microglia, macrophages and DC) that are most likely to be implicated in the effector phase of EAE. We therefore studied the susceptibility of pI-/- mice to EAE induced by active immunization with myelin oligodendrocyte glycoprotein (MOG) peptide 35-55, as well as by the adoptive transfer of MOG-reactive encephalitogenic CD4+ T cells. In both models, pI-/- mice were almost completely protected. In the active immunization model, MOG-specific T cell priming was essentially normal despite the fact that EAE did not ensue. Taken together these results demonstrate that the block in EAE development in pI-/- mice lies at the level of antigen presentation during the effector phase in the CNS. To determine whether the absence of MHCII expression by CNS resident microglia or blood-derived APC is responsible for the resistance of pI-/- mice to EAE, we generated reciprocal bone marrow-chimeras between wild type and pI-/- animals. Interestingly, both types of chimeric mice (pI-/- into wt and wt into pI-/-) developed EAE, whereas pI-/- into pI-/- control chimeras remained strongly protected. These results show that MHCII-restricted antigen presentation by both blood-derived and CNS-derived APC is involved during the effector phase of EAE, and that these two APC compartments can compensate for each other's absence.

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

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Rationale: Natural and inducible regulatory T cells (Tregs) are key players in controlling the development of asthmatic inflammation. However, the role of these cells in the mechanisms leading to tolerance in an established model of asthma has not yet been defined.
Methods: To examine the respective role of these subsets in the induction of allergen specific tolerance in established asthma, BALB/c mice were sensitized to OVA, tolerized intranasally with OVA and depleted of CD25+ cells by intraperitoneal injection of anti-CD25 mAb (PC61) during tolerance induction. Mice were then challenged by OVA aerosols and efficiency of tolerization was evaluated. T cell subsets were characterized by flow cytometry. Their suppressor activity and proliferation were determined by in vitro coculture systems and by cell transfers experiments.
Results: Intranasal treatment with OVA led to a marked upregulation of CD4+CD25+ Foxp3+ T cells in the lungs. These cells were regulatory as shown by their suppressive and anergic characteristics in vitro. CD25+ T cells depletion severely hampered tolerance induction as indicated by a strong recruitment of eosinophils into BALF and a vigorous T cell response to OVA upon challenge, in contrast to non depleted mice. However, 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. PKH26 labeled CD4+CD25- T cells did not proliferate in vivo, although they did proliferate in vitro. When stimulated with OVA in vitro, CD4+CD25- T cells produced high amounts of IL-10, low IL-5 and no TGF-b, IL-17 or IFN-g. They were equally distributed in the spleen, BLN and lungs.
Conclusions: In conclusion, both CD4+CD25+ and CD4+CD25- T cells appear to be essential in tolerance induction. The functional relationship between both subsets will have to be further analyzed.

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A novel population of human melanoma-specific CD8 T-cells recognises melan-A/MART-1 immunodominant non-apeptide but not the corresponding decapeptide

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HLA-A2-restricted cytolytic T cells specific for the immunodominant human tumor Ag Melan-A/MART-1 can kill most HLA matched melanoma cells, through recognition of two naturally occurring antigenic variants, i.e., Melan-A nonamer AAGIGILTV and decamer EAAGIGILTV peptides. Several previous studies have suggested a high degree of TCR cross-reactivity to the two peptides. In this study, we describe for the first time that some T cell clones are exclusively nonamer specific, because they are not labeled by A2/decamer-tetramers and do not recognize the decamer when presented endogenously. Functional assays with peptides gave misleading results, possibly because decamers were cleaved by exopeptidases. Interestingly, nonapeptide-specific T cell clones were rarely Vbeta2.1 positive (only 1 of 19 clones), in contrast to the known strong bias for Vbeta2.1-positive TCRs found in decamer-specific clones (59 of 69 clones). Molecular modeling revealed that nonapeptide-specific TCRs formed unfavorable interactions with the decapeptide, whereas decapeptide-specific TCRs productively created a hydrogen bond between CDR1beta and glutamic acid (E) of the decapeptide. Ex vivo analysis of T cells from melanoma metastases demonstrated that both nonamer and decamer-specific T cells were enriched to substantial frequencies in vivo, and representative clones showed efficient tumor cell recognition and killing. We conclude that the two peptides should be regarded as distinct epitopes when analyzing tumor immunity and developing immunotherapy against melanoma.

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Dendritic cell-activated, endothelial cell-specific CTL recognising a minor histocompatibility antigen rapidly induce transplant vasculopathy

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Occlusive coronary artery disease is the major cause for cardiac allograft rejection. Endothelial cells (EC) are the first graft cells encountered by host lymphocytes and are therefore primary targets of activated cytotoxic T lymphocytes (CTL). This study determined whether CTL-mediated injury of EC exclusively presenting a minor histocompatibility antigen (mhAg) would suffice to precipitate transplant vasculopathy (TV).The interaction of CTL with antigen-presenting EC in vivo has been examined here using a transgenic mouse model with beta-galactosidase (b-gal) expression confined to the vascular endothelium (Tie2-LacZ mice). Cardiac grafts from Tie2-LacZ mice were transplanted heterotopically into C57BL/6 recipients. EC-specific CTL were activated in vivo either by priming with b-gal peptide-pulsed dendritic cells (DCs) or by infection with b-gal-recombinant mouse cytomegalovirus (MCMV-LacZ). In the absence of b-gal-specific effector CTL, Tie2-LacZ heart grafts remained immunologically ignored for more than 100 days post transplantation. Repetitive priming with b-gal peptide-pulsed DCs elicited severe vascular inflammation in transplanted Tie2-LacZ hearts with neointima formation and vascular occlusion. Activation of EC-specific CTL by infection with MCMV-LacZ caused less severe vascular inflammation in Tie2-LacZ hearts presumably due to the timely limited activation of b-gal-specific CTL under these conditions. Taken together, EC injury mediated by activated CTL recognizing a mhAg specifically expressed on EC is sufficient to elicit TV. Prolonged antigen presentation within secondary lymphoid organs – most likely by DCs – appears to be a key factor for the development of chronic vascular rejection.

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E7-specific CD8 T-cell immune responses in the genital mucosa of mice vaccinated against HPV-16 and cervical cancer

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Background: Cervical cancer, the second leading cause of cancer mortality in women worldwide, results from an infection with a subset of human papillomavirus (HPV), HPV-16 being the most prevalent type. The available prophylactic vaccines are an effective strategy to prevent this cancer but decades will be needed before it may be eradicated. Therapeutic vaccines are necessary to eliminate infected cells and associated lesions in women who have no benefit from prophylactic vaccination. Until now, therapeutic vaccines only showed poor clinical results, possibly linked to an inefficient targeting of protective immune responses in the genital mucosa.
Methods: Mice were immunized via mucosal or parenteral routes with a synthetic HPV16 E71-98 polypeptide vaccine administered with different adjuvants. E7-specific CD8 T cell responses were evaluated by IFN-g ELISPOT in the blood, draining lymph nodes and in the genital mucosa.
Results: Parenteral vaccination with CpG and HLT adjuvants induced high E7-specific responses in the blood, whereas combination of Resiquimod, HLT and CpG was the best combination after an aerosol immunization, although this response was 5-fold lower than after the parenteral vaccination. The amplitude and kinetics of E7-responses were also measured in draining lymph nodes and more importantly in the genital tissue itself. Despite the lower E7-specific response in the periphery after the aerosol immunization, the responses measured in the genital mucosa after both types of vaccination were similar, suggesting a preferential genital homing after aerosol vaccination. Interestingly, there was no correlation between the responses measured in the periphery with those measured in the genital mucosa, highlighting the necessity to determine the immune responses in the mucosa where the tumor reside. Finally, we have evidences that additive applications of topical immunomodulators locally enhanced the therapeutic properties of the E71-98 polypeptide vaccine.
Conclusion: Our results suggest that combination of the adjuvanted E71-98 peptide with topical immunomodulators could be a potent therapeutic vaccine against HPV-16 and cervical cancer.

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Cytokine production by peripheral blood cells following therapy with a combined vaccine of allergen and QbG10

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Immunological sensitization to common environmental allergens results in diseases such as allergic rhinitis. Susceptible individuals develop allergen-specific T helper 2 (Th2) immune response characterized by IgE production, and secretion of IL-4, IL-5 and IL-13 from Th2 cells. We have developed a therapeutic vaccine to treat allergic diseases, which comprises an allergen extract and Qb virus-like particle (VLPs) filled with the immunostimulatory sequence (ISS) G10 (QbG10). A phase IIa clinical study has been conducted to explore the immunogenicity and the clinical efficacy of the allergy vaccine. The following study groups were included: i) QbG10+Pollen (QbG10 combined with grass pollen formulated in Alum); ii) QbG10 alone; iii) QbG10 adsorbed to Alhydrogel; iv) PBS and v) PBS mixed with Alhydrogel. PBMC isolated from the volunteers before and after treatment were stimulated with either medium or grass pollen extract for six days in vitro and IL-5, IL-10, IFN gamma and TNF alpha in the supernatants were measured using the flex set cytometric bead array (Becton Dickinson).
Among the tested cytokines, the Th2 cytokine IL-5 dominated the pollen-specific response of PBMC from allergic volunteers before treatment. Upon vaccination, a decrease of IL-5 was detected in the majority of the volunteers, regardless of the treatment. However, pollen-specific IL-5 was significantly decreased only following QbG10+Pollen therapy ($p = 0.006$). None of the other groups showed a significant reduction in pollen-specific IL-5. Furthermore, IFN gamma and TNF alpha were produced at very low levels and did not change upon treatment.
Interestingly, up to 70% of the study volunteers' PBMC released IL-10 following treatment with combination vaccine consisting of allergen and QbG10. As a result, the levels of IL-10 in this group significantly increased upon treatment ($p = 0.003$). The increase in IL-10 upon treatment with QbG10+Pollen was also statistically significant if compared to the change in IL-10 levels in both placebo groups (PBS – $p = 0.049$ and PBS in Alum – $p = 0.014$).
Taken together, these data suggest that treatment of allergic volunteers with a combined vaccine of allergen and QbG10 results in pollen-specific downmodulation of the Th2 cytokine IL-5 and upregulation of the regulatory cytokine IL-10.

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Inhibition of mediator release by designed ankyrin repeat proteins against the alpha chain of the high affinity receptor for IgE

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Background: In atopic disease the activation of mast cells and basophils by aggregation of the high-affinity receptor for IgE (Fc epsilon RI) results in the release of various inflammatory mediators. To prevent this degranulation either inhibitory anti-IgE or anti-Fc epsilon RI antibodies could be used. Several monoclonal anti-Fc epsilon RI alpha antibodies inhibiting the interaction of IgE with its receptor were described so far but most of them are anaphylactogenic.
Designed ankyrin repeat proteins (DARPins) represent a novel protein scaffold for high-affinity binding molecules that might be used as an alternative to antibodies. They consist of 33 amino acids long repeat modules stacking together to form a hydrophobic core and a large accessible surface for target binding. Combinatorial DARPin libraries with randomized interaction surfaces containing either two (N2C) or three (N3C) internal repeat modules have been generated.
Methods: DARPins against the extracellular part of Fc epsilon RI alpha were selected using ribosome display. N2C and N3C binders were characterized by ELISA and surface plasmon resonance and tested for inhibition of receptor cross-link induced degranulation using rat basophilic leukemia cells stably transfected with human Fc epsilon RI alpha (RBL-2H3-hu alpha).
Results: Selected binders recognized Fc epsilon RI alpha with high affinity and showed no cross-reactivity to any other tested protein. Two DARPins binding to different epitopes on Fc epsilon RI alpha were expressed as bivalent and bispecific constructs. We demonstrated that one of these bispecific DARPins prevents receptor cross-link induced degranulation of RBL-2H3-hu alpha cells. Moreover our bispecific DARPin inhibited mediator release in the same range as the humanized monoclonal anti-IgE antibody Omalizumab (Xolair®) which is currently used to treat moderate-to-severe asthma.
Conclusion: Our data demonstrated that anti-Fc epsilon RI alpha DARPins might represent a novel approach in the treatment of allergy as an alternative to Omalizumab. The therapeutic potential of a DARPin in vivo is not yet determined and has to be further evaluated.

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House dust mite avoidance: effect on FENO

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Background: Allergy to house dust mites is a main cause for perennial rhinitis and allergic asthma. It goes along with an elevated FENO level due to allergen contact and eosinophilic airway inflammation. To investigate the usefulness of recommendations for mite avoidance, we measured symptom scores, bronchial FENO, FEV_1, and blood eosinophils/ECP levels before and after mite avoidance procedures.
Materials and methods: 34 patients with mite allergy and FENO levels >35 ppb (normal levels <20 ppb) were invited to participate in an open, controlled trial. Recommended mite avoidance procedures were carefully explained and included removal of carpets from sleeping room, HEPA filter in the vacuum cleaner, weekly change of linen and low sleeping room temperature. Encasings for cover, pillow and mattresses (Allergocover, Allergomed, Therwil and ACb Comfort, Allergy Control Products, Trimedal, Brütisellen) were provided. The control group postponed these measures till the end of the study. The study parameters FENO (NiOX, Aerocrine, Trimedal), FEV_1, eosinophilia and ECP were measured twice before, and once at ca 10 weeks after start of the intervention. Additionally, the patients provided data on symptom scores for nasal and asthma symptoms and need for medication (only beta-mimetics and anti-histamines, steroid use was not allowed). Statistical analysis was done using one-sided Mann Whitney Test.
Results: 6 patients were excluded due to non-compliance, lack of specific IgE against mites, use of steroids and asthma exacerbations. Twenty eight patients terminated the study, 14 in the intervention (7 males) and 14 (9 males) in the control group. The parameters FENO ($p = 0.05$), asthma ($p < 0.001$) and rhinitis ($p < 0.001$) symptoms, medication use ($p < 0.025$) improved significantly in the intervention group, if compared to the start. If compared to the control group, significant reduced values were obtained for asthma ($p < 0.01$) and rhinitis ($p < 0.01$) symptoms and medication use ($p < 0.05$) but not for FENO. No difference regarding eosinophilia, ECP, or FEV_1 was observed.
Conclusion: Careful and extensive mite reduction procedures lead within 10 weeks to improved asthma and rhinitis symptoms; this improvement is also documented by a reduced FENO-level in the intervention group, which represents an objective parameter supporting the usefulness of mite reduction procedures in Switzerland.

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Secretory immunoglobulin A and mucosal homeostasis

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Background: An important activity of mucosal surfaces is the production of the special type of antibody referred to as secretory IgA (SIgA). SIgA serves as the first line of defense against microorganisms through a mechanism referred to as immune exclusion. In addition, SIgA has also the capacity to adhere selectively to M cells in intestinal Peyer's patches (PP), resulting in the transport of the antibody molecule across the epithelium back to the gut-associated lymphoid tissue.
Methods: Mice were given orally fluorescent SIgA and tissue sections were analyzed by laser scanning confocal microscopy. In parallel experiments, SIgA were used as carrier for protein and bacterial antigens in oral immunization.
Results: In the PP, SIgA binds and is internalized by dendritic cells (DC) in the subepithelial dome region. SIgA induced mucosal and systemic responses associated with production of anti-inflammatory cytokines and limited activation of dendritic cells. Immune complexes made of SIgA and enteric bacteria enter the mouse intestinal epithelium, yet preventing tissue destruction.
Conclusions: At mucosal surfaces, SIgA antibodies appear thus to combine properties of a neutralizing agent (immune exclusion) and of a mucosal immunomodulator inducing effector immune responses in a noninflammatory context favorable to the development and maintenance of local homeostasis of the gastrointestinal tract.

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Effect of oral Lactobacillus paracasei on allergic rhinitis in a nasal provocation test with grass pollen

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Background: Lactobacillus paracasei strain NCC2461 (ST11) has been found to exert anti-allergic effects in animal models.
Objective: To investigate the effect of a short-term oral administration of ST11 prior to a nasal provocation test (NPT) with grass pollen on manifestations of allergic rhinitis.
Methods: 31 subjects (18–35 years-old) were enrolled in a randomized, double-blind, placebo-controlled, cross-over study. The study consisted of a first 4 weeks period in which subjects consumed either ST11 fermented milk (ST11 group) or placebo (acidified milk) (placebo group), a washout period of six to eight weeks and a final 4 weeks period cross-over of the first treatment phase. The entire study was performed out of the pollen season. Clinical symptoms were analysed after a NPT using grass pollen allergens, and immunological parameters (specific immunoglobulins in serum, cytokines and differential cell count in nasal brushes and cytokines secreted by allergen stimulated peripheral blood mononuclear cells (PBMC)) were compared between the two treatment periods.
Results: No significant change of the nasal reaction threshold was observed between the end of the first and second treatment periods, whatever the sequence (active product before or after placebo) of product consumption. However, ST11 group declared having globally less congestion than placebo group (visual analogical scale; $p < 0.05$). Nasal pruritus followed the same trend. This result was associated with a lower secretion of IL-5 by PBMC stimulated with grass pollen in ST11 group as compared to placebo group ($p < 0.03$). IL-8 and IL-10 secretion by allergen stimulated PBMC followed the same trend. Finally, allergen specific IgG4 were significantly reduced in plasma, favouring probiotic consumption. No effect on mucosal samples was observed.
Conclusion: Despite a short term treatment prior to NPT, the effect of the fermented milk containing the L. paracasei NCC2461 (ST11) reached significance for a clinical marker (subjective nasal congestion) and for systemic immune markers (IL-5 and IgG4). This was supported by the non significant trend for a decrease of other markers such as IL-10 and IL-8 secreted by allergen stimulated PBMC and nasal pruritus.

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Severity of Hymenoptera venom allergy in relation to baseline serum tryptase levels

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Introduction: The association between elevated baseline tryptase (bT) and the grade of a systemic allergic reaction in Hymenoptera venom allergic patients is well established. Today a bT concentration higher than 11.4 µg/l (95th percentile of normal) is considered as elevated. According to a recent publication already values between 8 and 10 µg/l may indicate an increased risk for systemic reactions in patients with Hymenoptera venom allergy. Our objectives were to analyze the distribution of bT serum levels in relation to the severity of the allergic reaction – including large local reactions – in Hymenoptera venom allergic patients.
Patients und methods: 736 patients referred 2003 until 2005 with confirmed Hymenoptera venom allergy (history, diagnostic tests) and available bT were included. The bT was determined by the UnicCAP tryptase fluoroenzyme immunoassay (Pharmacia Diagnostic, Uppsala, Sweden). According to the bT, patients were assigned into the following groups: 639 (86.8%) had a bT <8 µg/l (mean 4.21; range 1.00–7.96), 48 (6.5%) had a value between 8 and 11.4 µg/l (mean 9.57; range 8.02–11.4), and 49 (6.5%) individuals had a bT >11.4 µg/l (mean 16.77; range 12.0–33.9).
Results: The distribution of different bT levels in the various severity grades is shown in Figure 1.
Conclusions: An elevation of bT was mainly observed in patients with systemic allergic reactions grade IV (according to H.L. Mueller). Interestingly, the incidence of an elevated bT in the grade III group was comparable with that in group I/II. This correlation could not only be observed in patients with values >11.4 µg/l but already in those with bT levels ≥8 µg/l.
Therefore, special attention should be drawn in patients with bT levels between 8 and 11.4 µg/l. If this association can be further confirmed, a decrease of the upper limit of normal values to 8 µg/l needs to be discussed.

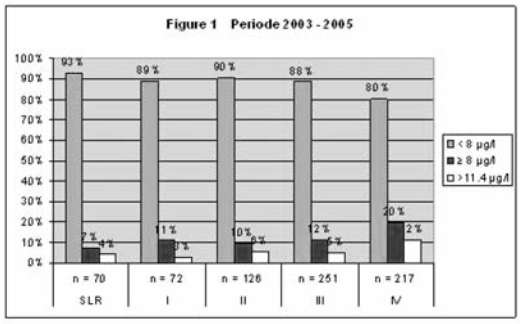

Table 1

basal serum tryptase	Total	Systemic allergic reaction 2003 - 2005				
	n = 736	SLR n = 70	I n = 72	II n = 126	III n = 251	IV n = 217
< 8 µg/l	639	65	64	114	222	174
		10.2%	10.0%	17.8%	34.7%	27.2%
≥ 8 - 11.4 µg/l	48	2	6	5	17	18
		4.2%	12.5%	10.4%	35.4%	37.5%
>11.4 µg/l	49	3	2	7	12	25
		6.1%	4.1%	14.3%	24.5%	51.0%
≥ 8 µg/l	97	5	8	12	29	43
		5.2%	8.2%	12.4%	29.9%	44.3%

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Usefulness of the basophil tests in monitoring the immune response to bee venom immunotherapy controlled by sting challenge

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Background: For life-threatening Hymenoptera venom allergy a 5 year-long specific immunotherapy with insect venom (VIT) is the treatment of choice. However, in 5 to 15% of VIT-treated patients no full immunologic protection can be achieved. As no reliable in-vitro marker for the evaluation of a successful VIT exists, a sting challenge with the relevant living insect is recommended before VIT is stopped. In this study we investigated whether a change in basophil activation and degranulation assays helps in identifying bee venom tolerant patients.
Method: 30 bee venom allergic subjects were examined. 20 subjects after VIT (just before sting challenge, group 1) and 10 subjects before starting VIT (group 2). Basophil tests were performed with commercially available kits (CAST = sulfidoleukotriene release, Flow CAST = anti-IgE/CD63 and Flow2 CAST = anti-CCR3/CD63, Buehlmann Lab) at varying in vitro conditions (± IL-3, whole blood vs. washed leukocytes). Basophils were incubated with various bee venom concentrations (2–10.000 ng/ml) to investigate whether subjects before or after VIT show a different dose response to bee venom.
Results: 19 of 20 VIT-treated patients tolerated the bee sting provocation. The bee venom concentrations ranging from 2 to 250 ng/ml were found to differentiate between subjects before and after VIT, while higher concentrations consistently activated basophils. The VIT-treated patients showed a significantly lower basophil activation and degranulation at a given concentration compared to patients before VIT (s. table).
Conclusion: A decreased basophil sensitivity could be demonstrated in bee venom allergic patients after VIT compared to patients before VIT. This finding correlates to the unproblematic sting challenge in 19/20 persons, suggesting that the higher concentration of bee venom to elicit a basophil activation or degranulation can serve as an in vitro surrogate marker for bee venom tolerance. The use of basophil activation tests with different bee venom concentrations is potentially able to identify those bee venom allergic subjects who need higher VIT doses or a longer duration of VIT for full protection.

Bee venom conc.	anti-IgE/CD63		anti-CCR3/CD63		CAST	
IL-3	+	-	+	-	+	-
250 ng/ml	p= 0.0031	ns	0.0061	ns	ns	ns
50 ng/ml	0.0009	0.0056	0.0003	0.0121	ns	0.0335
10 ng/ml	0.0003	0.0017	0.0018	0.0121	0.0252	0.0168
2 ng/ml	nd	nd	0.0455	0.0061	0.0098	0.0098

p values (Mann-Whitney-U-Test) ns=not significant nd=not done

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Canonical Wnt signalling is required for thymus organogenesis

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The thymus achieves two interrelated functions essential for the adaptive immune system; the life-long generation of new T cells and the selection of a repertoire of T cells tolerant to self-antigens but reactive to foreign peptides. Thymus organogenesis is initiated in the mouse at embryonic day 10.5 when endodermal cells of the third pharyngeal pouch become committed to a thymus fate. The formation of an epithelial primordium and the subsequent differentiation of thymic epithelial cells (TEC) into distinct subpopulations constitute the necessary prerequisite for the formation of a thymic microenvironment proficient to support T cell development. However, the precise molecular signals that dictate TEC fate commitment and differentiation remain largely unknown. Wnt signaling has, however, been implicated in thymus organogenesis Here we demonstrate that thymus organogenesis is differentially affected depending on the timing of the loss in canonical Wnt signalling. Deletion of canonical Wnt signalling during developmental stages prior to the formation of a thymus anlage causes thymic agenesis. In contrast, loss of canonical Wnt signalling at a later stage does not preclude the formation of a regularly structured and composed thymic microenvironment but leads to thymic hypoplasia despite normal T cell development. Taken together, these results demonstrate an essential role for canonical Wnt signaling during early stages of thymus organogenesis whereas activation through the same pathway determines thymus cellularity during later stages and organ maintenance of the thymus.

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Mature Aire+ mTEC development is controlled by antigen-specific TCR-MHC class II mediated interactions with autoreactive CD4+ thymocytes

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Medullary thymic epithelial cells (mTEC) are specialized for inducing central immunological tolerance to self-antigens. To accomplish this, mTEC 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 direct mTEC maturation are poorly understood. We have discovered that the number of mature mTEC is controlled in the postnatal thymus by direct physical interactions between CD4+ thymocytes bearing autoantigen-specific TCR and mTEC displaying the cognate self-peptide/MHC class II complexes. The competence of CD4+ thymocytes for this process is imparted by their expression of CD40L, which delivers an essential signal upon binding to CD40L on mTEC. This crosstalk between CD4+ thymocytes and mTEC defines a novel checkpoint pivotal for thymic stromal and lymphoid development because it generates a mature mTEC population competent for ensuring central T cell tolerance.

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Role of Notch1 and Notch2 receptors in CD4+ T helper differentiation

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Notch proteins are evolutionary conserved receptors involved in cell differentiation and development processes, especially in binary cell fate decisions. The role of the Notch pathway in T helper (Th) differentiation remains controversial. In order to characterize the role of Notch1 and Notch2 in CD4+ Th differentiation, we used mice with a T cell-specific ablation of the Notch1 (N1-/-), Notch2 (N2-/-) or both Notch1 and Notch2 (N1-/-N2-/-) and infected them with Leishmania mexicana, which induces IL-4-dependent cutaneous leishmaniasis and Th2 differentiation when injected in the back rump. Evolution of lesions and of the immune response was compared to that of their control littermates (+/+). Lesion development as well as parasite number within the lesions did not differ significantly within the infected groups, however, parasite metastasis was observed only in control groups but not in the Notch-/- groups. Fourteen weeks after infection with L. mexicana, control+/+ mice developed significant levels of L. mexicana-specific IgG1 and IgE antibodies indicating of the development of a Th2 immune response. In contrast, infection of N1-/- mice resulted in a decreased production of parasite-specific IgG1 and IgE in the serum, with corresponding increase in Th1-specific IgG2a antibodies. This decrease in Th2 antibodies with the corresponding increase in IgG2a antibodies was even more drastic when CD4+ T cells lacked both Notch1 and Notch2 (N1-/-N2-/- mice). In contrast, L. mexicana-infected N2-/- mice had similar levels of IgG1 and IgE as controls littermates but the secretion of IL-4 and IL-10 by CD4+ T cells was significantly enhanced while the secretion of IFN-g was decreased, suggesting a role of Notch2 in Th1 differentiation. Taken together, these results reveal that the IL-4 produced by CD4+ T cells is not essential for lesion development following infection with L. mexicana, but is involved in parasite metastasis. In this model of infection, Notch1 contributes to Th2 differentiation while Notch2 appears to be involved in Th1 response and both Notch1 and Notch2 seems to regulate each other.

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T-cell development occurs in the combined absence of the canonical Wnt signal-transducers beta- and gamma-catenin

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The canonical Wnt signaling pathway plays key roles in stem cell maintenance, expansion of committed progenitor cells and control of lineage decisions in a variety of tissues including the hematopoietic system. Beta-catenin is the central molecule in the canonical Wnt signaling pathway. Beta catenin transmits Wnt signals into the nucleus, where it acts as transcriptional co-activator by binding to members of the LEF/TCF (Lymphoid enhancer factor/T cell factor) family of transcription factors. The related molecule gamma catenin (plakoglobin) can fulfill similar functions to activate target genes upon Wnt signaling.
Gene ablation of TCF-1 impaired T cell development. TCF-1 deficiency resulted in incomplete blocks within the CD4-8- thymocyte compartment and at the transition from immature single positive to the CD4+8+ stage. Strikingly, a genetic complementation approach showed that the N-terminal domain of TCF-1 was essential to rescue thymocyte development in TCF-1 deficient mice. This domain in TCF-1 includes a beta catenin binding site. We show that this domain also contains a gamma catenin interaction site, suggesting that catenin binding to TCF-1 is critical for T cell development. However, results obtained by individual gene ablations of the two known signal transmitters beta- and gamma catenin did not recapitulate the phenotype of TCF-1-deficient mice.
Based on this discrepancy we analyzed thymopoiesis in the combined absence of both, beta- and gamma catenin. Unexpectedly, we find that T cell development occurs normally in the combined absence of the two known Wnt signal transmitters beta- and gamma catenin. We performed reporter assays to address whether canonical Wnt signaling was not needed for thymocyte development or whether such signals were transduced in the absence of beta- and gamma catenin. We find that beta- and gamma catenin-deficient thymocytes and peripheral T cells retain a significant capacity to transduce canonical Wnt signals. These data suggest a novel mechanism of TCF-1/Wnt signal transduction in the hematopoietic system.

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Interleukin-7-driven networking in normal and ectopic lymphoid organ development

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Antimicrobial adaptive immune responses are generated in secondary lymphoid organs such as spleen, lymph nodes (LNs) and Peyer's patches (PPs). In addition, inducible ectopic lymphoid tissues form in inflammatory lesions of chronic infections, autoimmune diseases, allergic reactions and chronic graft rejection. These so-called "tertiary lymphoid tissues" can function as inductive sites for adaptive immune responses against self and non-self antigens. During embryogenesis,

the development of secondary lymphoid tissues is regulated by the interactions of CD45+CD4+CD3- lymphoid tissue inducer (LTi) cells with mesenchymal VCAM-1-expressing organizer cells. Interleukin(IL)-7 is an essential survival factor for LTi cells. The fate of LTi cells in adults has been the subject of considerable debate. Here we show that in mice with increased availability of IL-7, high numbers of adult LTi cells are detectable in secondary lymphoid organs. They share the phenotype and function of fetal LTi cells and induce the formation of PPs after adoptive transfer into neonatal CXCR5-deficient mice, which lack PPs. Moreover, mice overexpressing IL-7 ubiquitously form additional secondary and tertiary lymphoid organs. The development and organization of these tertiary lymphoid organs is dependent on lymphotoxin alpha-beta and LTi cells. Our findings extend the previously defined role of IL-7 for fetal secondary lymphoid organ development to a role in controlling adult LTi cells and tertiary lymphoid organ development.

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The proteolytic activity of Malt1 plays a key role in T-cell activation

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The Malt1 protein plays an essential role in antigen receptor-mediated lymphocyte activation and shows altered expression or function in various types of lymphomas.
Stimulation of the T or B cell receptor leads to a PKC-dependent formation of the Carma1-Bcl10-Malt1 (CBM) complex. This complex is essential for optimal NF-kB activation and thus regulates key processes of lymphocyte activation like cytokine production and cell proliferation. Therefore components of the CBM complex represent attractive drug targets to treat patients suffering from autoimmune diseases, transplant rejection or lymphomas.
Malt1 contains a caspase-like domain, but so far it is unknown whether this domain is proteolytically active. Here we report that Malt1 has arginine-directed proteolytic activity which can be measured by a peptide based cleavage assay. Malt1 activity is markedly increase after T cell receptor (TCR) stimulation and is dependent on PKC activation and Carma1 oligomerization. We identify a peptide based inhibitor targeting Malt1 activity, which negatively regulates NF-kB activation and IL-2 production in T cells. Furthermore, we discovered the first Malt1 substrate which is cleaved upon TCR stimulation and constitutively processed in various forms of B-cell lymphomas.
Thus, the proteolytic activity of Malt1 plays a central role in T-cell activation, suggesting a novel target for the development of immuno-modulatory and anti-cancer drugs.

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TRAIL receptor-mediated Jun kinase activation and Bim phosphorylation critically regulates liver damage

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TRAIL (TNF-related apoptosis-inducing ligand) is a member of the TNF family with potent apoptosis-inducing activities in tumor cells. In particular, TRAIL strongly synergizes with conventional chemotherapeutic drugs to induce tumor cell death. Thus, TRAIL has been proposed as promising future anti-cancer therapy. However, little is known regarding the role of TRAIL in untransformed cells and whether therapeutic administration of TRAIL, alone or in combination with other apoptotic triggers, may cause tissue damage. In this study we have investigated the role of TRAIL in Fas (CD95/Apo-1)-induced hepatocyte apoptosis and liver damage. While TRAIL alone failed to induce apoptosis in isolated murine hepatocytes, it strongly amplified Fas-induced cell death. Importantly, endogenous TRAIL was found to critically regulate anti-Fas antibody induced hepatocyte apoptosis, liver damage and death in vivo. TRAIL enhanced anti-Fas-induced hepatocyte apoptosis through the activation of Jun kinase and its downstream substrate, the pro-apoptotic Bcl-2 homolog Bim. Consistently, TRAIL- or Bim-deficient mice, or wild type mice treated with a Jun kinase inhibitor, were protected against anti-Fas-induced liver damage. We conclude that TRAIL and Bim are important response modifiers of hepatocyte apoptosis and identify liver damage and lethality as a possible risk of TRAIL-based tumor therapy.

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Intestinal bacteria condition dendritic cells to promote IgA production

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Immunoglobulin A represents the predominant antibody isotype produced at the intestinal mucosa, where it plays an important role in limiting the penetration of commensal intestinal bacteria and opportunistic pathogens. We investigated the role of dendritic cells (DC) from different tissues of origin in the differentiation of IgA producing B cells in a co-culture system. We show in mice that Peyer's Patch-derived DC (PP-DC) exhibit a specialized phenotype allowing the promotion of IgA production. PP-DC exhibited increased production of the B-cell activating factor of the tumor necrosis family (BAFF) and a proliferation-inducing ligand (APRIL), which were necessary for IgA production in the absence, but not the presence, of CD40- or LPS-mediated signalling. In contrast, in the absence of BCR stimulation no IgA was detected when DC originated from peripheral lymph nodes. Furthermore DC presence was mandatory for IgA, but not IgG1 and IgM, production and IgM and IgG1 levels were comparable and independent of the origin of the DC. Intestinal bacteria are a unique feature of the mucosal immune system and are known to play a role in the mucosal immune system development. We investigated their role in the ability of PP-DC to instruct naïve B cells to differentiate into IgA producing plasma cells. For this purpose we compared IgA levels in co-cultures with DC from germ free or SPF mice. Interestingly, the presence of intestinal commensal bacteria largely accounted for the preferential IgA production. These data indicate that exposure to intestinal commensal bacteria, as well as other intestinal environmental factors, condition DC to promote IgA production.

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Double-stranded RNA induces apoptosis in spleen CD8a dendritic cells

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In the event of microbial invasion, dendritic cells (DC) are central to the detection and processing of pathogen in order to induce efficient adaptive immune responses.
The Toll-like Receptor (TLR) network is well known to mediate pathogen detection and potent activation of DC. Thereafter, evidence on the effect of TLR-ligands on the lifespan of DC is, however, contradictory and incomplete. While increased survival may enhance their function, apoptosis may serve to prevent over-activation leading to autoimmunity and to limit the spread of infection.
In the present project, we made use of novel splenic CD8a DC lines, derived from tumors in mice transgenic for the SV40 Large T oncogene under the DC-specific CD11c promoter, where CD8a DC preferentially transform. They are known to express TLR3 (detecting dsRNA during viral infection) and to be capable of antigen cross-presentation.
Stimulation of DC lines with PolyIC (synthetic TLR-3 ligand) induces potent activation, however, dramatic apoptosis is observed after 24-48 hours. We took advantage of these novel DC lines to characterize the apoptotic mechanisms involved and extended our studies to normal DC.
Altogether our results show upregulation of Bim as a key feature of DC apoptosis and an important role of type I interferon signaling for induction of cell death in DC lines and wild type DC.
These results provide clues for the optimization of the use of PolyIC and DC-based strategies for therapeutic purposes.

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Role of TLR7 and TLR9 in a murine model of SLE

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Background: Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disorder characterized by the formation of a variety of autoantibodies and subsequent development of immune complex (IC) glomerulonephritis, i.e. lupus nephritis. 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 role of Toll-like receptor 7 (TLR7) and TLR9 in the development of murine SLE has been suggested. Notably, the Yaa (Y-linked autoimmune acceleration) mutation, which markedly accelerates the progression of murine lupus, was identified to be a translocation from the telomeric end of the X chromosome – containing the gene encoding TLR7 – onto the Y chromosome.
Methods: To determine the contribution of TLR7 and TLR9 to the development of SLE, we introduced the tlr7 or tlr9 null mutation into C57BL/6 mice congenic for Nba2 (NZB autoimmunity 2) locus bearing the Yaa mutation (B6.Nba2.Yaa) and followed the development of the pathology.
Results: Males B6.Nba2.TLR7-/Yaa (i.e. bearing a single copy of TLR7) produced reduced serum levels of IgG autoantibodies (against DNA and ribonucleoproteins), and also incidence of lupus nephritis. However, the protection was not complete, since these mice still developed high titers of anti-chromatin and retroviral gp70-anti-gp70 immune complexes and severe lupus nephritis, which was not the case in B6.Nba2 male mice lacking the Yaa mutation. In contrast, mice B6.Nba2.TLR9-/-.Yaa displayed an accelerated development SLE (50% mortality at 5 month of age vs. 14 month in B6.Nba2.Yaa controls) with an enhanced development of autoimmune responses and an increased TLR7-dependant B cell and plasmacytoïd dendritic cell activation.
Conclusion: Our results indicate that the Yaa-mediated acceleration of SLE cannot be totally explained by the Tlr7 gene duplication alone, and that TLR7 and TLR9 play an opposing role on the development of SLE.

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CD4+ and CD8+ T-cells exhibit differential requirements for CCR7-mediated antigen transport during influenza infection

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Upon encounter of viral antigens in an inflammatory environment, dendritic cells upregulate costimulatory molecules and the chemokine receptor CCR7, the latter being pivotal for their migration to the lymph node. By utilizing mice deficient in CCR7, we have examined the requirement of DC-mediated antigen transport from the lung to the draining lymph node for the induction of anti-influenza immune responses in vivo. We found that CCR7-mediated migration of DC was more crucial for CD8+ T cell than CD4+ T cell responses. Whilst no specific CD8+ T cell response could be detected in the airways or lymphoid tissues during the primary infection, prolonged infection in CCR7-deficient mice did result in a sustained inflammatory chemokine profile, which led to non-specific CD8+ T cell recruitment to the airways. The recruitment of influenza-specific CD4+ T cells to the airways was also below levels of detection in the absence of CCR7 signaling, although a small influenza-specific CD4+ T cell population was detectable in the draining lymph node, which was sufficient for the generation of class-switched anti-influenza antibodies and a normal CD4+ T cell memory population. Overall, our data show that CCR7-mediated active antigen transport is differentially required for CD4+ and CD8+ T cell expansion during influenza infection.

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Restricted B-cell receptor diversity converts acute into chronic LCMV infection

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Persistent infections like HIV and HCV represent a great burden to global health but preventive vaccines remain unavailable. Cytotoxic T cell responses are key for virus control, but recent results from clinical HIV trials suggest that coordinate action with other immune mediators may be needed for vaccine protection. Accordingly, the contributive role of specific antibodies in resolving chronic virus infections has recently gained interest.
Here, we studied the contribution of virus-specific B cell responses to control of lymphocytic choriomeningitis virus (LCMV) infection in mice, which is also predominantly mediated by cytotoxic T cells. To circumvent the drawbacks intrinsic to B cell-deficient mouse models (i.e. disorganized splenic microarchitecture, impaired CD4+ T cell responses) we exploited a panel of genetically engineered B cell-competent mice with differentially restricted B cell receptor (BCR)

repertoires. Thus, virus-specific CD4+ and CD8+ T cell responses were normal, but their antiviral activity was not or only weakly supported by viral glycoprotein-specific antibody responses. We found that virus control correlated strictly with the animal's antibody repertoire diversity and thereby with the capacity to mount LCMV-GP-specific antibodies. Severe restriction in BCR diversity even resulted in conversion of acute into chronic infection.
Our results highlight the importance of virus-specific B cell responses in supporting CTL-mediated virus control, and in preventing viral persistence. This has important implications for refining future vaccine strategies against persisting viruses like HIV and HCV.

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Leishmania major induces secretion of inflammatory cytokines and chemokines by keratinocytes via a TLR2 pathway

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Some features of the cutaneous pathologies observed in patients with cutaneous leishmaniasis can be reproduced in the murine model of infection with L. major. After subcutaneous injection of parasites in C57BL/6, mice are resistant to infection; in contrast BALB/c mice are susceptible to infection. Resistance and susceptibility to the infection were related to the development of polarized Th1 and Th2 response. Keratinocytes are the first effector cells encountering parasites during infection with Leishmania major (L. major) but their role in the initiation of the immune response is mainly ignored. The aim of this study is to analyse the role of the interaction between L. major and keratinocytes in the subsequent immune response induced by infection with this parasite.
Cultured primary murine keratinocytes from neonatal C57BL/6 and BALB/c mice (1–3 days old) were stimulated in vitro by L. major LV39 strain (MHRO/Sv/59/P strain), and mRNA expression and secretion of inflammatory cytokines (IL-1, IL-6 and TNF-a) and chemokines (MIP-2, KC) were analyzed.
Parasites adhere to keratinocytes through the flagellar tip, the flagellar base or with the posterior pole in both strains of mice, but they were not shown to infect keratinocytes. This interaction induces an up-regulation of IL-1, MIP-2 and KC mRNA expression and secretion in both BALB/c and C57BL/6 mice, however, IL-6 is produced in response to L. major only in BALB/c mice. Furthermore, we demonstrated that MIP-2 and KC secretion are abolished in TLR-2-/- C57BL/6 keratinocytes and partially reduced in TLR-4-/- C57BL/6 keratinocytes. Moreover, we identified leishmania lipophosphoglycan (LPG) as a parasitic molecule responsible of MIP-2 and KC secretion by C57BL/6 keratinocytes via the TLR-2 pathway. The results strongly suggest that keratinocytes are able to produce chemokines in response to L. major stimulation mainly through activation of TLR-2 and then may influence immune responses towards this parasite.

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Junctional adhesion molecule C (JAM-C) constitutes a new diagnostic marker for B-cell lymphoproliferative syndromes

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Differentiation of naïve B cells into plasma cells or memory cells occurs in the germinal centers (GC) of lymph follicles or alternatively via a GC- and T cell independent pathway. It is currently assumed that B cell lymphomas correlate to normal B cell differentiation stages, but the precise correlation of several B cell lymphomas to these two pathways remains controversial.
We recently described the junctional adhesion molecule C (JAM-C), a molecule originally identified at the cell-cell border of endothelial cells, as a new B cell marker with a tightly regulated expression during B cell differentiation: immature CD10+ B cell in the bone marrow do not express JAM-C; naïve, mature, peripheral blood B cells express it weakly; CD27+ memory B cells strongly; and bone marrow plasma cells are again JAM-C negative (Leukemia; 2007 Jun; 21(6):1285-93). Of particular interest, JAM-C expression divides CD27+ tonsillar B cells into two subpopulations: JAM-Cneg cells, with a phenotype of germinal center B cells and a high expression of BCL-6, a nuclear proto-oncogene with a pivotal role in GC-formation, and JAM-Cpos cells, corresponding to non-germinal B cells, derived partly from the marginal zone. In vitro cultures of the different tonsillar B cell subpopulations (CD27+Jam-C+, CD27+JAM-Cneg, CD27negJAM-C+) confirmed these results, since Ig-secretion measured after 7 days of culture, was minimal in naïve CD27neg cells, CD27+Jam-C GC cells produced mainly IgG, and non-GC JAM-C+CD27+ B cells mainly IgM.
Simultaneous analysis of JAM-C and CD27 expression in peripheral blood lymphocytes (PBLs) from a series of 86 patients with various lymphoproliferative syndromes (LPSs) allowed a clear classification into two types of B cell malignancies: JAM-Cneg lymphomas (CLL, follicular lymphoma, DLBL, multiple myeloma, and B-ALL), and JAM-C expressing lymphomas (mantle cell lymphoma, marginal zone B cell lymphoma, hairy cell leukemia). In 13 LPSs a clear diagnosis could not be obtained using classical surface markers, immunohistology, and cytogenetic analyses, in particular in cases on the borderline between MCL, CLL and MZBL. The use of JAM-C in the diagnostic work-up of these cases will be discussed.
In conclusion, we suggest that JAM-C constitutes a new diagnostic marker for the characterisation of lymphoproliferative B cell syndromes, and in particular for the positive diagnosis of lymphomas derived from the marginal zone.

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Heterozygous hypomorphic STAT3 mutations in 4 Swiss patients of 3 unrelated families with classic autosomal dominant hyper IgE syndrome

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Background: Hyper-immunoglobulin E syndrome (HIES) is an autosomal dominant disorder characterized by a highly elevated serum IgE, eczema, recurrent staphylococcal skin abscesses and cyst-forming pneumonia, with disproportionately milder inflammatory responses, referred to as cold abscesses, and skeletal abnormalities. Therapy involves lifelong antibiotic and antimycotic prophylaxis, and occasionally surgical abscess drainage. The molecular defect, heterozygous mutations of STAT3 has recently been identified
Methods: We enrolled 4 patients from 3 unrelated families with the classic symptoms of HIES and looked for mutations in the gene encoding human signal transducer and activator of transcription 3 (STAT3) by sequence analysis.
Findings: We found hypomorphic heterozygous STAT3 mutations in all four patients with HIES suggesting a dominant negative effect. We identified a novel R335W mutation within the DNA-binding domain in two related patients from Sri Lanka, and a V637M mutation in the other two non-related Swiss patients. The V637M mutation is one of the four known STAT3 hotspot mutations and is located within the SH2 domain. There was no difference in the clinical phenotype between patients with mutations in the DNA binding or SH2 domain.
Conclusion: For four decades the molecular basis of HIES has remained elusive. Here we show that dominant-negative mutations in the STAT3 gene result in classic multisystem HIES in four patients followed in Switzerland. The identification of STAT3 as the major causative gene of HIES will facilitate early and definitive diagnosis as well as treatment, hopefully leading to the prevention of serious infectious complications and sequelae.

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Tumour antigen specific CD8+ T-cells from melanoma patients exert strong ex-vivo cytolytic activity

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Direct analysis of ex vivo cytotoxicity by tumor Ag-specific T-cells has long been precluded, because standard laboratory methods are not suited to analyze small numbers of T-cells available from PBMC and tumor infiltrated lymph nodes (TILN) from cancer patients. Here we applied the recently described LiveCount Assay which allows to determine the cytotoxic function of small numbers of isolated T-cells. We analyzed Melan-A/HLA-A2 tetramer+ CD8+ T-cells from eight melanoma patients. Surprisingly, strong cytotoxic activity was exerted by T-cells derived from TILN or PBMC isolated after peptide vaccination. Contrary to previous reports showing that TILN produce low to no tumor-specific responses in vitro, our results illustrate that they can be quite effective if isolated from the tumor microenvironment, and thus separated from suppressor and/or regulatory cells that might be contained therein. T-cells isolated from TILN showed a trend to display lower killing activity than those from PBMC, though the difference was not significant. As expected, concomitant measurement of specific lysis and degranulation revealed that only a subset of the cells were responsible for the observed strong cytotoxicity. A direct correlation was observed between the proportion of CD107a+ T-cells that were potentially involved in the killing activity and the frequency of Granzyme B+ T-cells detected ex vivo, although not all Granzyme B+ cells participated in the lytic activity. We conclude that tumor antigen specific T-cells are highly functional outside of the tumor microenvironment. Studies are underway to elucidate the factors that repress the effectiveness of tumor-specific T-cells locally, and how to promote activation and avoid inhibition of protective immune mechanisms.

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Depletion of B-cells by rituximab therapy improves atopic eczema

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Background: Atopic eczema (AE) is a chronic inflammatory skin disorder characterized by eczematous skin lesions, pruritus and typical histopathological features. Although T cells play a key role, B cells are also found in the dermal infiltrate. In 80% of AE patients, elevated total and specific IgE levels can be detected. This study aimed to investigate the effect of a monoclonal anti-CD20 antibody therapy (rituximab) in AE.
Methods: Six patients (2 males; mean age 39 ± 7 years) with severe AE received two applications of rituximab, each 1000 mg IV two weeks apart. To evaluate the efficacy of rituximab, we monitored clinical parameters (EASI, pruritus), total and specific IgE levels, and skin histology. Inflammatory cells and cytokine expression in skin lesions were assessed by immunofluorescence analysis before and after therapy.
Results: All patients showed an improvement of their skin symptoms within 4 to 8 weeks. The EASI significantly decreased (before therapy: 29.4 ± 4.3; week 8: 8.4 ± 3.6; $p < 0.001$). B cells were undetectable in the peripheral blood within 3 days. In contrast, IgE levels did not significantly change as a consequence of rituximab treatment. However, histological alterations, such as spongiosis, acanthosis, and dermal infiltrate, dramatically improved. B cell and T cell numbers were reduced by 50% in the skin. Skin cytokine expression, in particular of IL-13, also declined.
Conclusion: Treatment with rituximab reduces skin inflammation in AE resulting in clinical improvement.

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Expansion of circulating NKG2D+ T-cells and expression of NKG2D-ligand MIC in granulomaous lesions in Wegener's granulomatosis

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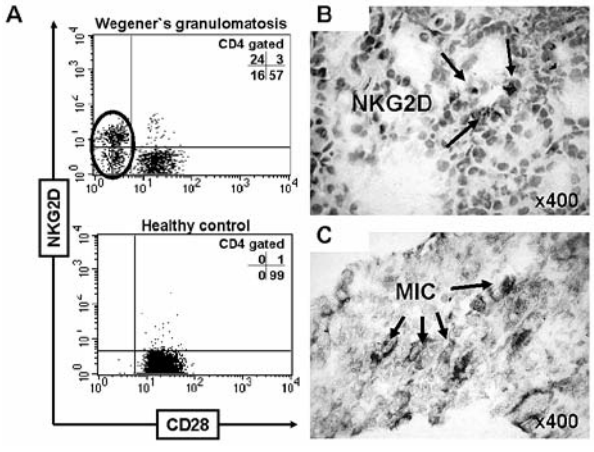
Introduction: Wegener's granulomatosis (WG) is a chronic inflammatory and autoimmune disease of as yet unknown etiology, characterized by granuloma formation and a systemic autoimmune vasculitis associated with highly specific antineutrophil cytoplasmic autoantibodies targeting "Wegener's autoantigen" proteinase 3 (PR3-ANCA). Expansion of circulating CD28- Th1-type cells reminiscent of effector memory T-cells (TEM) has been reported in WG recently. To investigate the role of CD28- T-cells in WG, we analyzed the expression of the activating NK-receptor NKG2D and its stress-inducible ligand MHC class I related molecule (MIC) on circulating TEM and in granulomatous lesions.
Methods: Flow-cytometric and immunohistochemistry analysis of NKG2D and MIC expression on circulating T-cells and in WG-granulomata, respectively. Patients / controls: 20 WG-patients / 10 healthy controls / 5 disease controls (chronic unspecific sinusitis, biopsies only).
Results: NKG2D was anomalously expressed and preferentially detected on circulating CD4+CD28- T-cells in WG. Circulating CD4+CD28- T-cells displayed a more activated phenotype compared to healthy controls. Cluster-like formations of PR3 were surrounded by NKG2D+ and MIC+ cells in WG-granulomata, but not in disease



controls. Interleukin (IL)-15 - known to drive TEM differentiation and proliferation and MIC expression in inflamed tissues – was also expressed in WG-granulomata. Serial sections showed sections with CD4+ cells matched sections displaying NKG2D positivity, and MIC+ cells with CD208 (dendritic cell marker) and IL-15 positivity.
Figure: A. Expanded circulating CD4+CD28-NKG2D+ T-cells in WG. B. and C.: NKG2D+ and MIC+ cells in WG-granulomata.
Conclusion: WG-granulomata display features suggestive of ectopic lymphoid tissue formation with PR3 clusters surrounded by NKG2D+ T-cells and MIC+ dendritic cells. Through acquisition of NK-like "innate" properties, IL-15 stimulated NKG2D+TEM could interact with MIC+ cells in WG-granulomata, thereby sustaining chronic inflammation and autoimmunity to "Wegener`s autoantigen" PR3 in WG.

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Ex-vivo generation of cancer/testis antigen specific cellular immune response from NSCLC TIL or healthy donors PBL

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Objective: Cancer/testis antigens (CTA) represent promising targets in different types of cancer. In lung tumors their expression correlates with short disease specific survival. We investigated expression of immuno-modulatory markers, CTA expression and specific CTL induction in freshly excised NSCLC specimens. Furthermore, since CTA immune responsiveness requires strong immunogenic reagents, comparative immunogenicity of a multi-CTA epitope recombinant vaccinia virus as well as the antigen presentation capacity of dendritic cells (DC) generated in the presence of IFN-alpha (IFN-DC) was evaluated.
Methods: Expression of MAGE-A family members and of NY-ESO-1 CTA was analysed by immunohistochemistry and quantitative real-time RT-PCR (qPCR). Expression within the tumor of factors known to influence immune response was also characterized by qPCR. Tumor infiltrating lymphocytes (TIL) were expanded from surgically removed and enzyme digested NSCLC samples. CD8+ cells obtained from HLA-A1+ and/or HLA-A2+ NSCLC patients' TIL or healthy donors PBL were stimulated in vitro with autologous mature DC obtained after IL4/GM-CSF (IL4-DC) or IFN-alpha/GM-CSF stimulation of monocytes. These cells were pulsed with soluble peptides or infected with a recombinant vaccinia virus encoding 6 CTA epitopes together with the human genes CD80 and CD154 (rVV-CGA). Phenotypes and functional CTL responses from TIL were evaluated by flow-cytometry and cytoxicity assays.
Results: CTA gene expression was detected in 15/33 NSCLC samples (45.5%). In 10/15 (66.7%), at least 4 CTA genes were concomitantly expressed. Despite frequent antigen expression, CTL responses were only detected in 1 patient and were specific for MAGE-A10. This lack of responsiveness was not associated with expression of IDO, FOXP3 or IL10 genes. However, effective CTL responses specific for MAGE-A10, a multi-MAGE epitope and NY-ESO1 could be more frequently generated using large numbers of CD8+ obtained from HLA-A2+ healthy donors after stimulation with recombinant vaccinia virus infected APC or with peptide pulsed IFN-DC.
Conclusion: CTA specific immune responses can be elicited from lung cancer cultured TIL or even from PBL of healthy donors using highly efficient immunogenic reagents, such as recombinant vaccinia virus or IFN alpha treated DC. These data strongly support the development of immunotherapy protocols for NSCLC.

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PFAPA-syndrome registry: analysis of a cohort of 214 patients

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PFAPA (Periodic fever, aphtous stomatitis, pharyngitis and cervical adenitis) syndrome was first described in 1987. The diagnosis is based on criteria, including unspecific symptoms and the exclusion of other periodic fever syndromes. Increased knowledge of the clinical and laboratory features of PFAPA may be useful to precise the diagnostic criteria.
With the purpose to investigate the clinical spectre, the clinical course and long-term follow-up of this entity, we established a web-based multicentric registry as an international collaboration within the working party "periodic fevers" of PReS. Patients, with PFAPA syndrome according to the previously published criteria, were included by filling-up a questionnaire with clinical data, laboratory values and treatment.
From November 06 to November 07, we included 214 patients with PFAPA from 14 centres and 8 countries: (122 males and 92 females; median age at onset 1.9 year; median age at diagnosis 4.0 years). The most prevalent clinical manifestation of the inclusion criteria was pharyngitis (94%) followed by cervical adenitis (83%) and aphtous stomatitis (59%), and 48% of the patients presented all 3 clinical features. 170 patients presented additional symptoms (gastrointestinal symptoms 131, arthralgias and/or myalgias 86, arthritis 4, genital aphtosis 5, skin rash 36, neurological symptoms 8 and splenomegaly 5). In 79 patients (37%) a genetic testing was done for periodic fever syndromes (FMF 49, TRAPS 52, HIDS 46, CAPS 7) and was negative, except for 8 cases (polymorphisms: 3, carrier for MEFV mutation: 5) without known clinical significance. Improvement or remission was observed in 99/105 patients with steroids, in 28/35 patients with tonsillectomy and in 5/15 patients with cimetidine.
We describe the largest cohort of PFAPA patients presented so far. We confirm that PFAPA syndrome may present with varied clinical manifestations and that the definition of the disease needs to be improved. Based on a detailed analysis of the data of this cohort, a new definition of PFAPA with better-defined criteria will be proposed and discussed in an international consensus conference. As follow-up to this retrospective study, we will follow prospectively a cohort of PFAPA patients to evaluate the long-term outcome.

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Exovesicles from human activated dendritic cells are phagocytised by epithelial cells, fused with endosomal compartments and stimulate the release of chemokines

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Dendritic cells (DCs) are able to release membrane vesicles, called exovesicles that are able to activate resting DCs and spread allo-antigens (AJPathol. 2006:169, 2127). Currently, we are interested in the role that exovesicles from DCs play in innate and adaptive immunity regarding the capacity to fuse with epithelial cells (ECs), to be internalized through endosomal compartments and allow ECs to present antigens.
Composition analysis of exovesicles by immunolabeling have shown that exovesicles harbor molecules from MHC-II, CD83 and CD40 as well as they are important TNF-a carries as shown by western blot. For functional studies human epithelial A549 cells (ECs) were labeled with a fluorescent lipophilic tracer and cultured with labeled exovesicles isolated from pre-stimulated LPS DCs. Cells were visualized with laser scanning microscopy. Results have shown that at an early time (1 h) exovesicles were rapidly internalized by ECs. Some exovesicles were processed through endosomal compartments observed by the colocalization of exovesicles and transferrin receptors. The release of inflammatory proteins was assayed by the bioplex array system. The results have shown that exovesicles from LPS-DCs are able to induce the release of chemokines by ECs, such as IL-6, IL-8, G-CSF, TNF-a, MCP-1, RANTES, GRO-a and in low concentration MIP-1b. Using TNF-a blocking antibodies the release of IL-8, G-CSF, MCP-1and TNF-a was blocked in a dose response manner.
We have shown for the first time that exovesicles from activated DCs are internalized by ECs through an endocity pathway in turn activating ECs to the release chemokines. Chemokine activation seems to be partially via TNF -mediated pathway, pointing out the key role of exovesicles, from DCs, as immunological tools triggering the innate response, activating ECs to recruit inflammatory cells.
Support for this work was provided by SNF grants 3140-107659-1 and 3200-065352.01.

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Bcl-2 over-expression in mice inhibits lung epithelial cell death induced by hyperoxia

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Background: Bcl-2 is an anti-apoptotic molecule preventing oxidative stress damage and cell death. We have previously shown that Bcl-2 was able to prevent hyperoxia-induced cell death in L929 transfected cells. We hypothesized that its specific over-expressed in pulmonary epithelial type II cells could prevent hyperoxia-induced lung injury.
Results: In murine pulmonary epithelial cells (MLE12), overexpression of Bcl-2 significantly protected cells from oxygen-induced apoptosis, as shown by measurement of LDH release ($p < 0.001$) and detection of Annexin-V positive cells. Bcl-2 interfered with mitochondrial pro-apoptotic signaling pathway: by decreasing the release of cytochrome c and inhibiting caspase 3 activation ($p < 0.001$). We concomitantly generated transgenic mice over-expressing Bcl-2 in epithelial type II cells under SP-C promoter (Tg-Bcl-2) and exposed them to oxygen during 72 hours. Lung damage was identical in both mice strains as measured by lung weight and BAL protein content. Nevertheless, we observed a reduced number of TUNEL positive cells and mitochondria of type II cells were preserved compared to littermate.
Conclusion: Our results demonstrate that the overexpression of Bcl-2 is able to prevent hyperoxia-induced cell death in epithelial type II cells in vitro and in vivo. However, its overexpression in type II cells was not sufficient to decrease acute lung damage in mice.
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Endothelial progenitor cell transmigration is dependent of inflammatory mediators

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Endothelial progenitor cells (EPC) released from bone marrow into the vasculature are considered as reparative cells for vascular lesions within the body. We could recently show that extracorporally expanded EPC uniquely homed in the injured lung or in a pro-angiogenic subcutaneously administered extracellular matrix (Matrigel) when they were intravenously injected ("transplanted") in rats after unilateral pulmonary ischemia-reperfusion injury by left-sided lung transplantation (Kahler CM et al., Respir Res 2007;8:50). The aim of the current study was to assess mediators possibly accounting for a homing using an ex-vivo/ in vitro transmigration assay as a model for homing of EPC.
EPC were collected from femurs of male Sprague-Dawley rats (220–280 g) using a Lymphoprep density gradient, then washed and suspended in EBM-2 medium (Lonza, Verviers, Belgium) supplemented with 20% foetal calf serum and plated on rat-derived fibronectin-coated (10 ìg/ml, Sigma) 12-well plates as described (Kahler CM et al., Respir Res 2007;8:50). The non-adherent cell population was daily carded and cultured in EBM-2 MV medium for two days. After another 2–3 days a kind of angioblast-like cells were observed and spindle-shaped cell outgrowth documented that was positive for von Willebrand factor, CD31, oxidized LDL- uptake, CD133, and VEGF-R2. Transmigration assays were performed with $5x10^4$ EPC in EBM-2 medium without additives per insert that were seeded on 8 µm microporous transwells (BD) in 24 well plates for 10 h; counting was performed by microscopy.
Bronchoalveolar lavage (BAL) was performed with 5 ml EBM-2 medium without additives in the left lung of a Sprague-Dawley rat after 45 min. of ischemia by clamping of the hilar vessels and three hours of reperfusion (I/R). The supernatant was used for the experiments; control lavages were performed in rats without ischemia-reperfusion injury.
Compared to control BAL (or medium alone), transmigration of EPC was significantly increased after 10h by BAL from the I/R lung by a factor of 3.9 (SD 0.6; $p < 0.001$). Pharmacological or neutralization experiments revealed that transmigration was corticosteroid and cyclooxygenase-2 dependent, and dependent of VEGF, of TNF, of CXCL 12 (stromal cell derived factor-1) and CXCR4 ($p < 0.05$ each versus BAL from I/R lung).
In conclusion, different inflammatory mediators influence transmigration of endothelial progenitor cells towards BAL supernatant from ischemia-reperfusion injured lung.

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Enhanced allergen-specific uptake and processing by dendritic cells and other antigen-presenting cells precedes the development of allergic airways disease

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Background: In the airways antigen-presenting cells (APC) and mucosal dendritic cells (AMDC) unremittingly scrutinise inhaled antigen and fine-tune the nature of the T cell-mediated immune response. While harmful pathogens cause immunity, non-pathogenic substances induce tolerance in healthy individuals. In allergic respiratory disorders such as allergic asthma, this homeostatic process is disturbed, causing a potentially damaging Th2-biased CD4+ T-cell inflammatory response to intrinsically non-pathogenic allergens.
Methods: We have investigated the functional changes occurring in AMDC and other airway APC populations during disease onset in a mouse model of experimental allergic airways disease (EAAD).
Results: Early but transient activation of airway CD4+ T cells coincided with an increased CD40 expression exclusively seen on CD11b- AMDC during the onset of EAAD. Simultaneously enhanced uptake and processing of allergen occurred within all airway APC populations, including B cells, macrophages, and both CD11b+ and CD11b- AMDC subsets. Immune serum transfer into naive animals recapitulated the enhanced allergen uptake observed in airway APC populations and caused activation of naive allergen-specific, airway CD4+ T cells following inhaled allergen challenge.
Conclusions: Onset of EAAD is initiated by enhanced allergen uptake and processing in airway APC populations. Allergen-specific immunoglobulins play a role in the conversion of normally quiescent AMDC subsets into those capable to activate airway CD4+ T-cells. The role of antigen-specific immunoglobulins in the development of allergic airways disease may have been hitherto underestimated and could provide the basis for novel therapeutic strategies.

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Fifteen years of lung transplantation in patients with Cystic fibrosis (CF): the Zurich experience

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Background: Cystic fibrosis (CF) accounts for one third of lung transplantation (LTx) performed in Zurich. There is an ongoing worldwide debate on whether LTx improves survival in CF, especially in younger patients (NEJM, 2007 Nov 22;357(21):2143–52).
Aim: To analyze LTx survival of CF patients in our transplant program and to characterize pre-LTx parameters which could influence LTx survival.
Methods: All CF patients transplanted between 11/1992 (start of our LTx program) and 11/2007 were included. The following parameters were analyzed: Age at LTx, date of LTx, gender, body mass index (BMI), diabetes status (DM), bone mineral density (T-score) of hip (FN) and lumbar spine (LS), pre-LTx FEV1 and Liou-Raw-Score (Am J Epidemiol, 2001). Univariate and multivariate Cox regression analysis were used. Post LTx 5-year survival was compared with calculated 5-year survival without LTx according to the model of Liou.
Results: 80 (35%) of the 231 LTx were performed for CF. Mean age at LTx was 26.2 years (95% CI 24.4–28.0, range 12.3–51.8), 39 (49%) female and 11 (13.8%) younger than 18 years. Pre-LTx FEV_1 was 830 ml (95% CI 770–890), 27% (24–29) of predicted. BMI was 17.3 kg/m^2 (16.7–18.0), FN-T-score –2.2
(–2.0- –2.4) and LS-T-score –2.8 (–2.5- –3.1). 33 (41%) patients had osteoporosis at FN and 49 (61%) at LS. Thirty-seven (46%) had DM at time of LTx. Liou-Raw score at LTx was –20 (–16- –24), resulting in an estimated 5-year survival without LTx of 33 ± 14% compared to post-LTx 5-year survival of 67±6%. 1-, 3-, 5- and 10-year survival after LTx in the Zurich CF population was 86 ± 4, 73 ± 5, 67 ± 6 and 60 ± 7%, respectively. In the 53 CF (66%) patients transplanted since 1/2000, 1-, 3- and 5-year survival was 90 ± 4, 80 ± 6 and 69 ± 7%, respectively. Results of univariate Cox regression analysis are summarized in Table 1 and indicate that LTx performed more recently (since 2000) and diagnosis of DM positively influenced post-LTx-survival, while young age (<18 y) had no negative impact on outcome. In multivariate analysis, only DM (more frequently diagnosed in later years) influenced post-LTx-survival (p = 0.02), in a positive manner.
Conclusion: In our lung transplant program survival after LTx for CF is better compared to the International Registry and 5-year survival with LTx is much higher compared to survival without LTx calculated according to the Liou-model. We could not find a negative influence of age at LTx on survival whereas later time of LTx and DM seemed to positively influence survival.

Table 1
Model: univariate proportional hazards

	Exp(Coef)	95% Lower	95% Upper	p(Wald)
Year of LTx*	0.867	0.768	0.977	0.02
DM**	0.311	0.116	0.834	0.02
FN-T-score	0.612	0.365	1.024	0.06
Age at LTx	0.948	0.896	1.003	0.07
BMI	1.084	0.967	1.214	0.16
Liou Raw score	1.016	0.994	1.038	0.16
LS-T-score	0.806	0.581	1.118	0.19
Female gender	1.202	0.538	2.685	0.65
FEV1 % pred	1.007	0.956	1.061	0.78

* Later LTx-date favours better outome after LTx
**DM favours better outcome after LTx

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Aspergillus-PCR in bronchoalveolar lavage for detection of invasive pulmonary aspergillosis

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Invasive pulmonary aspergillosis (IPA) is a frequent and serious infectious complication in neutropenic patients. Despite the introduction of voriconazole and other new antifungal agents (Herbrecht N Engl J Med 2002;347:408–15) the mortality remains as high as 30%. There is suspicion of IPA if neutropenic patients develop antibiotic resistant fever and pulmonary infiltrates. Typical features of IPA on CT scan include the halo sign or cavities. However, in many cases there are only unspecific infiltrates. Culture and cytology in BAL have a high specificity but very low sensitivity for the diagnosis of IPA as assessed by histological confirmation of the diagnosis (Reichenberger et al BMT 1999;11:1195–1199). In our experience, serum galactomannan is not helpful for the diagnosis of IPA in neutropenic patients (Weisser M et al Clin Inf Dis 2005;41:1143–1149). We therefore developed conventional and quantitative PCR to detect aspergillus fumigatus, flavus, niger, glaucus, terreus and tomarrii. BAL samples of 143 patients with pulmonary infections requiring bronchoscopy were analysed. There were 50 hematologic patients (leukaemia 35, lymphoma 15) undergoing high dose chemotherapy with or without autologous or allogeneic stemcell transplantation, 36 immunocompromised patients for other reasons (18 solid organ transplantion, 6 HIV, 12 autoimmune disease) and 22 patients with primary lung diseases (asthma, bronchiectasis, cystic fibrosis, ABPA). Based on clinical data, CT scan and histology 2 patients suffered from proven, two from probable, 13 from possible and 33 from no IPA as defined by international guidelines. There was aspergillus colonisation or ABPA in 7 cases. Using the fast protocol there were 42 positive PCR cases (29.4%), quantitative PCR revealed a positive result in 24 cases respectively. A total of 33 cases were positive by both nested PCRs or quantitative PCR (23%). Sensitivity, specificity, positive predictive value and negative predictive value for proven or probable IPA in hematologic patients were 75.0%, 78.3%, 23.1% and 97.3% respectively.
Summary and conclusion: Aspergillus-PCR in bronchoalveolar lavage might be helpful to better diagnose invasive pulmonary aspergillosis in immunocompromised patients. Different PCR protocols are currently evaluated to define optimal test conditions.

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Breathing disturbances in obstructive sleep apnoea patients at high altitude

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Background: Many patients with obstructive sleep apnea syndrome (OSA) travel to high altitude for leisure time and professional activities despite potentially adverse effects on their health. We evaluated the hypothesis that high altitude exposure agravates sleep related breathing disturbances and promotes central sleep apnea in OSA patients.
Methods: 16 OSA patients residing at <600 m discontinued longterm CPAP therapy for 8–9 days. They spent days 1–4 at low altitude and days 5–9 at high altitude (Davos Schatzalp, 1850 m, and Davos Jakobshorn, 2590 m, 2 nights each). They were randomized to undergo polysomnography a), at 490 m (Zurich) in the night before ascent to altitude and at 2590 m (Davos Jakobshorn) in the last night at high altitude, or b), in the last night at 2590 m (Davos Jakobshorn) and after return to 490 m (Zurich). Sleepiness and acute mountain sickness were evaluated in the morning by the Stanford and the Lake-Louise questionnaires. Data from nights at 490 m and 2590 m were compared.
Results: At 490 m, patients had predominantly obstructive apnea/hypopnea (table). At 2590 m, the total number of events and the fraction of central events had increased. Symptoms assessed by the Lake Louise score and subjective sleepiness were similar at low and high altitude.
Conclusions: Sleep related breathing disturbances in OSA patients are more pronounced at high altitude than at low altitude and the apnea/hypopnea are predominantly central rather than obstructive. These findings may have therapeutic implications for OSA patients at high altitude.
Funded by LungenLiga Zurich

	490m	2590m
AHI total (1/h)	41±24	76±22*
AHI obstructive (1/h)	37±23	13±21*
AHI central (1/h)	4±5	63±23*
central/obstructive AHI	0.12±0.17	107±160*
SpO2%	94±2	86±4*
Lake Louise Score	3.1±2.1	3.8±3.1
Standford Sleepiness Score	4.1±2.0	3.9±2.0
means±SD; *P<0.001 vs 490m		

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Drops in pulse wave amplitude, a micro-arousal scoring surrogate

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Introduction: During sleep, sudden drops in pulse wave amplitude are commonly observed simultaneously with microarousals. Their presence is thought to result from a vasoconstriction induced by an autonomic central nervous system activation. We sought to determine if pulse wave amplitude drops are associated with cortical activation as quantified by EEG spectral analysis.
Methods: EEG spectral analysis was performed over 5 consecutive epochs of 5 seconds before, #1+2: during #3 and after # 4+5 the pulse wave amplitude drops (>20%). A total of 1084 events, from 10 consecutive sleep polygraphic recordings were analysed. The presence or absence of visually scored EEG arousals was also determined (according to AASM criteria). EEG spectral analysis was performed over five wave lengths: (beta 17-30 Hz, alpha 8–12 Hz, theta 4-8 Hz, sigma 12–16 Hz and delta). The power density of each type of EEG wave was compared between the five epochs using repeated measures ANOVA with a Tukey post hoc test.
Results: The global analysis of all drops in pulse wave revealed a significant increase in EEG power density of all EEG wave for the epoch #3 in comparison to the preceding (#1-2) and subsequent (#4-5) ones ($p < 0.001$). Further analysis of pulse wave drops not associated with a visually recognized microarousal also revealed a significant increase in EEG power for all types of waves during the pulse wave drops (epochs #3; $p < 0.001$).
Conclusion: Pulse wave amplitude drops, observed on polygraphic sleep recordings, are associated with a sudden increase in EEG power density in all wave length. This suggests that drops in pulse wave amplitude are concomitant to central nervous system activation, even in absence of microarousal.

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Respiratory research funded by the Swiss Respiratory Society: is it worth the money?

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Through an unrestricted grant from the Swiss Lung League, the Swiss Respiratory Society (SRS) is able to offer annual funding for one to three research projects in our country. The research committee of the SRS is responsible for the selection process of the grant proposals. The purpose of this study was to evaluate the scientific impact in term of publications of the funded projects.
Data were extracted from SRS archives and publications related to the selected projects and applicants were searched on PubMed. Only original articles, but not reviews nor book chapters were considered. Impact factor for each article was found on ISI Web of Knowledge. From 2000 to 2006, 18 projects get funding from the SRS. Research topics include pulmonary cell biology (n = 4), lung transplantation (4), sleep medicine (3), asthma or COPD (2), and others (5). The total allocated sum was CHF 681'400 with a mean (SD) of CHF 37'860 (23'340) per project. Fourteen published articles were found clearly related to the selected projects. All but one were published in international respiratory journals. The projects without any publications yet were submitted from 2004 and thereafter. The median impact factor of the 14 articles was 5.167 (range 1.005–9.091). The mean time between funding and publication was 1.8 year. We conclude that despite the relatively modest amount of money allocated to each project, the overall quality of the research, based on bibliometric data is excellent. It is assumed that for the searcher this funding is a valuable adjunct to national grant agencies.

P112

Phylogeographical lineages of Mycobacterium tuberculosis isolated in Switzerland between 2002 and 2006 as defined by spoligotyping

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Background: Recent evidence suggests that the human pathogen Mycobacterium tuberculosis (Mtb) has a clonal genetic population structure that is geographically constrained and that genetic variability of Mtb may translate into phenotypic differences, such as differences in virulence and immunogenicity. This study used spacer oligonucleotide typing (spoligotyping) to analyse the relative distribution of the major phylogeographical lineages among Mtb isolates cultured in Switzerland between 2002 and 2006.
Methods: Mtb patient isolates that were deposited in the strain repositories of the Universities of Basle and Berne in the years 2002 to 2006 were included in the study. DNA was extracted from cultures, and spoligotyping was performed using a spoligotyping kit (Isogen Life Science, Ijsselstein, NL). Spoligo-International-Type (SIT) numbers were assigned using data published in SpolDB4. Data were compiled in Microsoft Access and analysed with the Mycobacteria MIRU-VNTRplus online analysis tool (http://www.miru-vntrplus.org).
Results: A total of 534 Mtb-complex isolates (Mtb, n = 518 (97%); M. bovis, n = 13 (2%); and M. africanum, n = 3 (1%)) were deposited during the study period. Of these, the spoligotyping patterns of 162 Mtb isolates were available for further analysis. The relative distribution of the four main phyleogeographical lineages (L1-L4) was: L1, 3.1% (n = 5); L2 ("Beijing"), 8.6% (n = 14); L3, 6.8% (n = 11); L4, 64.2% (n = 104). Overall, 21 (13%) Mtb isolates had orphan spoligotyping patterns; in addition, 2 (1.2%) and 5 (3.1%) isolates were of SpolDB4 lineages S and U, respectively.
Discussion: Most (64.2%) of the Mtb isolates so far studied were attributable to L4. In addition, 13% of the spoligotype patterns were orphan and could not yet be attributed to one of the four major phylogeographic lineages. The relative contribution of L2, i.e. "Beijing" strains, was 8.6%. Since in Switzerland two out of three tuberculosis (TB) cases occur in foreign-born persons, the combining of spoligotyping data with country of origin and socio-demographic patient information as well as drug susceptibility testing (DST) results will be necessary to delineate the existence of "autochthonous" spoligotypes and to study the impact of foreign strains on the epidemiology of TB in Switzerland. This will require the combination of spoligotyping with additional typing methods, such as mycobacterial interspersed repetitive unit-variable-number tandem repeat analysis (MIRU-VNTR).

P113

Use of interferon-gamma release assays (IGRA) vs. tuberculin skin testing for detecting latent tuberculosis (TB) in chronic haemodialysis patients

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Background: IGRA have been shown to be more sensitive and more specific than tuberculin skin testing(TST) for the detection of latent tuberculosis infection in immuno-competent individuals and in HIV infected subjects. Their efficacy in other immuno-suppressed patients such as those under chronic hemodialysis (CH) is not yet well documented.
Methods: Prospective inclusion of patients under CH, with simultaneous sampling of 2 IGRA (T-SPOT.TB, Oxford Immunotec, UK and QuantiFERON-Gold in tube: QFT, Cellestis, Australia), measurement of TST, questionnaire, incidence in country of origin, history of prior exposure or treatment for TB, BCG status, and review of chest X-rays for signs of prior TB infection.
Results: 62 patients (16F, 46M, aged 65 ± 15 years, 50% foreign-born, 10% originating from high incidence countries, 5 with previous TB) were included; TST was >5 mm in 12 (19%), >10 mm in 9 (14%); T-SPOT.TB was positive in 18 subjects (29%, 6 indeterminate); QFT was positive in 14 cases (22%; 3 indeterminate). Agreement between IGRA was 73% (kappa value: 0.58; 95% CI: 0.34–0.82). Agreement between TST (>10 mm) and both IGRA was low (kappa: 0.23; 95% CI: 0.0–0.48 for T-SPOT.TB and 0.20; 95%CI: 0.0–0.48 for QFT). Multivariate logistic regression showed that increasing age was associated with a decrease in probability of having a TST >10 mm. A history of prior contact with TB was predictive of a positive QFT (OR: 10.3; 95% CI: 1.5–69.3; p = 0.016), and a TST >10 mm (OR: 32.7; 95% CI: 1.9–564; p = 0.016); the trend was not significant for T-SPOT-TB (OR: 14.7; 95% CI: 0.8–277; p = 0.07). None of the other variables analysed were predictive of results of either IGRA or TST.
Among 5 patients with a history of prior TB, TST and T-SPOT.TB were positive in 1, and QFT, in 2/5.
Conclusion: Both IGRA identified more CH patients with probable LTBI or prior TB than the TST, suggesting that they may be more sensitive than the TST. Agreement between IGRA was good, but agreement between both IGRA and TST was low. The fact that most patients with prior TB were not identified by either test illustrates however the limits of available tools for reliably detecting prior TB infection in CH patients.

P114

Tolerance to rifampicine (RIF) 4 months vs. isoniazid (INH) 6 months for latent tuberculosis infection (LTBI): a case-control study

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Background: Recent international guidelines have recommended INH for 9 months as first-line treatment for LTBI. RIF for 4 months is an interesting alternative, because of a shorter duration of therapy, and potentially a lesser hepato-toxicity. In our centre, RIF has become the default treatment for LTBI as of 2004. We aimed to compare the frequency of increase of ASAT and/or ALAT 3 x and 5 x above maximal normal level, completion rate of treatment for LTBI, and rates of interruption of treatment because of side effects, between subjects treated by INH for 6 months (ad 2003) and subjects treated with RIF 4 months (as of 2004)
Methods: Case-control study based on a retrospective analysis of all patients treated for LTBI by INH (1993–2002) or RIF (2004-7); database including age, gender, history of pre-existing liver disease or alcohol consumption, completion rates, time and causes of interruption, as well as monthly analysis of ASAT, ALAT and compliance (urinary INH).
Results: 636 subjects were included (48% F, 52% M, aged: 33.2 ± 11.4 years), 429 treated by INH, and 207 by RIF. Age, gender and history of prior liver disease (1.6 vs 1.9%) did not differ between groups. Reported alcohol consumption (5.0% vs 1.4%, chi2; p = 0.01) was slightly higher in the RIF group. Treatment interruption (17.6%) was related to non-compliance for 10.7% and side effects for 6.9% of all patients.
When controlling for age, gender, history of prior liver disease and alcohol consumption, occurrence of increase of ASAT and/or ALAT 3x or 5x above upper limit of normal did not differ between groups (logistic regression; 4.4% with RIF vs 5.8% with INH; p = 0.437). Clinical hepatitis occurred in 3 patients with INH and 1 with RIF. Probability of interrupting treatment (logistic regression, with age, gender, history of prior liver disease and alcohol consumption as

covariates) was much lower with RIF than with INH (OR: 0.32; 95% CI: 0.19–0.54). Interruption of treatment because of side effects increased with age (OR: 1.03, 95%CI: 1.01–1.06, p <.001) but did not differ between INH and RIF. Interestingly, interruption for non compliance was much lower with RIF than with INH (OR: 0.09; 95% CI: 0.03–0.26, p <.001).
Conclusions: In this study, the use of RIF was associated with a better compliance than INH. Signs of biological hepatitis did not differ between groups. Age was associated with an increase in treatment interruption because of side effects, independantly of treatment choice.

P115

Contact tracing for tuberculosis (TB): not always that simple!

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Background: Control of TB in developed countries relies mainly on screening of high risk groups and on contact tracing of subjects exposed to contagious forms of TB in order to detect and treat latent tuberculosis infection (LTBI) and, in 1–2%, active TB. Most cases of TB, in the Geneva area, occur in foreign-born subjects. Previous studies in Switzerland have shown that clusters are rare, most cases of TB resulting from reactivation of prior infection acquired in country of origin (O Philippin, Thesis N°10190, Geneva, 2001; GE Pfyffer et al; Eur Respir J 1998; and Bull OFSP 2002;9:175).
Setting: In a specialized centre for screening and treatment of TB in Geneva (CAT), annual surveillance statistics showed, in 2006, an unexplained over-representation of patients originating from Thailand among TB cases (n = 6), when compared to the 5 previous years. Review of our database showed that, during the 2004-6 period, 13 cases of active TB occurred among Thai subjects (foreign-born subjects represented 83% of our TB cases). All subjects had been interviewed by a trained social worker and had volunteered the names of a total of 101 contacts (average: 7.8 contacts per case, range: 0–42), all of whom were screened at the CAT. Tuberculin skin test was >10 mm in 42/101 cases (41%); no case of active TB was detected. None of the active cases had been previously screened as contacts.
Methods: In order to detect a possible link between these cases, in spite of the apparent lack of relationship established during the contact tracing inquiry, genotyping (spoligo-typing) of the 13 strains of M tuberculosis was performed; 11 subjects were infected by the same strain, belonging to the "Beijing family" of M tuberculosis; 2 other subjects had different un-related strains. Contacts were made with community leaders to understand the difficulty in obtaining reliable information among this particular community: it appeared that TB was particularly stigmatising – more than HIV – and thus the reluctance of patients to inform other members of the Thai community was enormous.
Conclusion: In this cluster of 11 cases, a centralized surveillance of the origin of TB cases in our area identified a link between cases which would have been completely missed by usual contact tracing inquiries. Strategies adapted to health representations and beliefs of different communities must be implemented; systematic genotyping may prove very useful to identify as early as possible unexpected clusters.

P116

Tuberculosis and latent tuberculosis infection among undocumented migrants in Lausanne

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Background: Undocumented migrants (estimated at 20'000 for 1 million inhabitants in Western Switzerland) are a potential source of tuberculosis (TB) and latent tuberculosis infection (LTBI) but are hard to reach and most of them have no insurance coverage. The presence of dedicated structures with access to care for undocumented migrants facilitates the approach to this population group.
Objectives: 1) To assess the prevalence of LTBI within a population of urban undocumented patients visiting two healthcare centres using a specific test (interferon-g) and 2) assess the adherence to preventive and curative treatment, if prescribed.
Method: All consecutive patients newly attending two healthcare centres for undocumented migrants staffed with nurse practicioners in Lausanne, between Jan and July 2007 were offered a screening for TB and LTBI using a questionnaire on risk factors and current symptoms and interferon-g test (T-Spot.TB). Participants with a positive T-SPOT.TB or suspect symptoms had a Chest X-ray and a medical examination. Compliance with therapy for TB or LTBI was evaluated at monthly visits at the tuberculosis department.
Results: Among 161 undocumented migrants visiting for the first time one of the two centres, 131 (81.4%) agreed to be screened and 125 had a complete examination. Most of the patients (83.2%) had entered in Switzerland without passing the official screening procedure for tuberculosis for asylum seekers and refugees. A positive T-Spot.TB test was observed in 19.2% [CI 95% 12.7–27.2] and active TB in 1.6% [CI 95% 0.2–5.7]. From the 18 patients with LTBI, 4 did not show up at the second visit. Ten patients had an indication for preventive therapy but 4 interrupted their treatment before the scheduled end.
Conclusion: Screening for active and latent tuberculosis in a hard to reach population is possible in dedicated structures. The prevalence of TB and LTBI in this population is high. The compliance with preventive therapy needs to be improved.

P117

Diagnosis of respiratory viral infections in the Institute for Infectious Diseases (Universitiy of Berne) during the last 5 years

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Rapid diagnosis of respiratory viral pathogens is important to prevent the spread of nosocomial infection, to minimize the use of unnecessary antibiotics and to discern the need of a possible antiviral therapy. Direct fluorescent-antibody assay (DFA) is a rapid, inexpensive and sensitive method for the detection of the most common respiratory viruses in respiratory specimens and is used in many clinical virology laboratories.
The discovery of new respiratory viruses in the last seven years allow us today, to diagnose viral respiratory infections that remained undiagnosed in the past. The detection of these new respiratory viruses is currently done by sensitive and expensive molecular methods but cheaper monoclonal antibodies for DFA are also available.
We tested from January 2003 until December 2005 all nasopharyngeal aspirates (NPA) from patients with respiratory infection for adenovirus, respiratory syncytial virus, parainfluenza virus 1-3 and influenza virus A and B by DFA. In addition we started in January 2006 to detect some of the new viruses (as the human metapneumovirus) by DFA. We analysed retrospectively the DFA-data of all NPAs submitted from patients (prevalently children) with respiratory infections during the period from 1st January 2003 until 31st December 2007. We analysed by DFA a total of 5238 NPAs. The positivity rate was 29.6% in 2003, 30.1% in 2004, 34.1% in 2005, 51.1% in 2006 and 51.4% in 2007. 100 frozen DFA negative NPAs were also retrospectively tested by xTag Respiratory Viral Panel (RVP) (Luminex Molecular Diagnostics) that detects additional coronaviruses and entero-/rhinoviruses. 46% of these samples were positive by this new multiplex PCR method.
Conclusions: Around 50% of NPA are actually positive by DFA in our clinical virology laboratory. This positivity rate could be increased to 70–75% by the use of RVP multiplex PCR but also at increased cost for the patients.

P118

Timely detection of multidrug-resistant Mycobacterium tuberculosis directly from smear-positive respiratory specimens

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Background: The world-wide emergence of drug-resistant Mycobacterium tuberculosis (Mtb) strains compromises the efficacy of modern tuberculosis (TB) drug regimens, of which rifampicin (RMP) and isoniazid (INH) are considered the pillars. Resistance to both drugs in Mtb is chromosomally determined by missense mutations and/or deletions. The novel GenoType MTBDRplus assay (HAIN Lifescience GmbH, Nehren, Germany) is designed to detect genetic events that are associated with INH and RMP resistance directly from smear-positive respiratory specimens, and thus allows the timely identification of multidrug-resistant Mtb (MDR-TB).
Methods: Mtb patient isolates and the corresponding sediments of smear-positive respiratory specimens were selected from our repositories. Drug susceptibility was assessed by the radiometric method (Bactec 460-TB instrument, PRISE, Becton-Dickinson, Germany). The critical concentrations in mg/L were: RMP, 2.0; INH, 0.1 and 0.4. GenoType MTBDRplus was performed according to the manufacturer's instructions, in a blinded manner, from DNA obtained from both the strains and the sediments.

Results: Ten Mtb isolates were evaluated. Of these, four had a MDR-TB phenotype, two were high-level INH-resistant, two were low-level INH-resistant, and two were susceptible to INH and RMP. There was a 100% agreement, in this collection of 10 Mtb isolates, between standard drug susceptibility testing and GenoType MTBDRplus results; in addition, the results of the GenoType MTBDRplus assay obtained from the strains and from the respective sediments were concordant. The turn-around-time of the GenoType MTBDRplus assay was approximately 5 hours.
Conclusions: These results indicate that GenoType MTBDRplus may have a role to play in the rapid detection of MDR-TB in the respiratory specimens of patients with suspected MDR-TB, e.g. with a relapse or coming from regions with a known MDR-TB risk. The assay's performance will depend upon the nature of the genetic events leading to INH or RMP resistance in the Mtb strains encountered in a particular clinical setting.

P119

A 24-yr old asylum seeker with advanced multidrug-resistant (MDR) cavernous lung tuberculosis

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Introduction: A 24-yr old man from the Ivory Coast came to Switzerland (CH) in early 2007 seeking asylum and treatment for his lung tuberculosis (TB). In his home country TB had been treated for several months. There, due to poor TB control measures and interrupted drug supply, drug resistance developed. Since 2005, asylum seekers in CH are no longer screened for TB by chest-XR or tuberculin test, but only interviewed by a specially trained nurse. First results on the efficacy of this new policy were published last year (1). Our patient's TB was first declared as extensively drug-resistant TB (XDR-TB) by a laboratory in Western CH. To date, XDR-TB has not been reported in CH.
Methods: Treatment was started in a local hospital according to the suggested drug resistance. After ten weeks of treatment, smears became negative. According to CH law, the patient was transferred to a shelter for asylum seekers in Lucerne. Due to administrative problems no specific measures of TB control were implemented. After several weeks sputum smears became positive again and the patient was sent to the Kantonsspital Luzern, from where he was transferred to the Luzerner Höhenklinik Montana.
Results: According to the most recent international recommendations the case was diagnosed as MDR-TB, since the strain was sensitive to moxifloxacin (MFX) and to amikacin (AMK), but resistant to all other first-line anti-TB drugs, except ethambutol (EMB). Treatment was established with INH, EMB, AMK, linezolid and MFX. After nine weeks, lobectomy of the left upper lobe was performed. Smears and cultures became and remained negative. After five months AMK and linezolid were stopped and treatment continued with INH, EMB and MFX. Treatment will be continued for at least one year.
Conclusions:
1. MDR-TB and XDR-TB should be defined according to the most recent WHO guidelines. Patients assumed to have an XDR-TB may «merely» suffer from an MDR-TB if their TB strain is susceptible to quinolones.
2. MDR-TB has a very poor clinical outcome in low income countries, whereas in Western countries most of these patients can be cured.
3. The information in CH between the Federal authority and the Cantonal authorities should be improved, especially in cases involving multiple drug resistant TB to avoid treatment interruptions and consecutive infections of others.

(1) Mathez Ch et al. Active screening for pulmonary tuberculosis by chest
x-ray among immigrants at the Swiss border. Swiss Med Wkly 2007;137:
649–54.

P120

Fungal pleural effusion as first manifestation of Candida albicans spondylodiscitis – a case report and review of the literature

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A 36-year-old male presented with increasing dyspnoea, orthopnea and left-sided thoracic pain. The patient reported a 10 kg weight loss in two months and night sweats,but no fever, cough or sputum production. The HIV-negative patient is known for intravenous drug abuse and untreated chronic hepatitis C. Physical examination revealed dullness on percussion and reduced breath sounds in left lower zone of the thorax. In addition lateral chest compression percussion of the thoracic spine was pain sensitive. The Body mass index was 18 kg/m^2. There were no radicular pain, neurogenic claudication or stigmata of chronic liver disease present. A computed tomography scan of the thorax was performed which revealed a pleural effusion on the left side and also demonstrated a destruction of the base of the thoracic vertebra Th 10/11. Cultured biopsy material from both the discus (Th 10/11) and from the pleural effusion revealed Candida albicans, which confirmed the diagnosis.
Candida spondylodiscitis of the thoracic spine is not uncommon in i.v. drug abusers, but is often diagnosed with delay. Symptoms such as severe back pain, fever or malaise, which are more typical for bacterial infections, are often less prominent in fungal spondylodiscitis.
In contrast, a candida albicans empyema is very rare. Several cases have been reported but the combination of effusion with candida albicans as main pathogen and fungal spondylodiscitis due to candida albicans has to our knowledge not previously been reported. The patient responded well to i.v. and subsequent p.o. antifungal treatment and was discharged 4 weeks later.

P121

Prevalence of anxiety and depression in patients freshly diagnosed with pulmonary hypertension

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Background: Pulmonary hypertension (PH) is a chronic disorder leading to a reduced quality of life. The cardinal symptom for patients in PH is exercise induced dyspnea, but patients often report feelings of anxiety and depression. The serotonin system has been described to be involved in the pathogenesis of PH and depression. We therefore investigated the prevalence of anxiety and depression during initial PH-evaluation and the frequency of serotonin-transporter (5HTT) and –receptor (5-HTR2A) polymorphism and their relation to functional parameters and hemodynamics
Methods: Consecutive patients diagnosed with PH by right heart catheterisation were included. Further study assessments were the hospital anxiety and depression score (HADS), demographics, NYHA functional class, 6 minute walk distance (6MWD), blood drawl for NT-pro-brain natriuretic peptide (pro-BNP), uric acid and genetic analysis for 5HTT(S/L) and 5HTR2A(C102T) polymorphism.
Results: 15 patients (10 females, age 69 ± 7) classified as PAH (7), CTEPH (4) and PH associated with chronic hypoxia (3) in NYHA class II/III/IV(2/4/9) were included. Mean pulmonary artery pressure was 45±13 mm Hg, PVR 737 ± 422 dyn*s*m-5, 6MWD 377 ± 132, pro-BNP 2385 ± 3380 ng/l and uric acid 426 ± 100 µmol/l. 30% of PH patients suffered from anxiety respectively depression, with mean HADS scores of 6 ± 4 respectively 6 ± 5. Patients with depression were slightly older (74 ± 5 vs 66 ± 6, p = 0.02) but otherwise comparable to patients without depression. Patients with anxiety tended to be younger (65 ± 7 vs 71 ± 7, p = ns) and were fitter than unaffected patients (6MWD 507 ± 117 vs 313 ± 84 m, p = 0.003; PVR 406 ± 222 vs 870 ± 132 dyn*s*m-5, p = 0.024). Polymorphism in the 5HTT and 5HTR2A were found heterozygote in 57 resp 42% and homozygote in 7 resp 36% of PH-patients (general population 45 resp 55% and 20 resp 22%) and were not correlated to anxiety, depression or other parameters of disease severity.
Conclusion: One third of patients diagnosed with PH suffer from anxiety and depression. However, in our collective both mood disorders were not related to other parameters of PH disease severity or polymorphism in the serotonin system. All the same patients with PH should be assessed for mood disorders as correct treatment may substantially affect patients well being.

P122

Vessel morphology of pulmonary arteries in COPD-patients with pulmonary hypertension: association between the caveolin-1 expression pattern and mean pulmonary arterial pressure (mPAP)

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Objective: End-stage chronic obstructive pulmonary disease (COPD) is commonly associated with pulmonary hypertension. Here we investigated the vessel morphology and expression of Caveolin-1 (Cav-1) in pulmonary arteries from COPD-patients that underwent lung-volume reduction surgery (LVRS).
Methods: Paraffin-embedded lung tissue sections were obtained from COPD-patients undergoing LVRS. Immunhistochemical staining with antibodies directed against Cav-1 was performed on serial sections, and staining was assessed by a visual scoring system (0 = no staining, 1 = rare, 2 = moderate, 3 = abundant). Morphometric data were obtained by using a computer-assisted imaging software (Axiovision, Zeiss). Pulmonary arteries with an external diameter of 100-500um were analysed (n = 7 per each patient) in order to outline the adventitial layer, external and internal elastic laminas as well as the intimal layer. For statistical analysis, the Mann-Whitney-test and correlation analyses (Pearson r) were used. A p value <0.05 was considered to be statistically significant.
Results: In COPD-patients with pulmonary hypertension, the ratio between the area of the smooth muscle cell layer and the area of the intima was found to be increased (1.31 ± 0.28) as compared to COPD-patients without concomitant pulmonary hypertension (0.77 ± 0.09). This ratio was significantly correlated to pulmonary hemodynamics, thus showing a linear increase with rising mPAP ($r2 = 0.51$, $p = 0.006$). Similarly, the thickness of the adventitial layer was strongly correlated to the mPAP ($r2 = 0.7$, $p = 0.0004$). The expression of Cav-1 within the smooth muscle cell layer was found to be upregulated in COPD-patients with pulmonary hypertension. On the other hand, the intimal Cav-1 was most prominently expressed in COPD-patients without pulmonary hypertension, but appeared to be downregulated in patients with pulmonary hypertension. Taken together, a significant correlation was found between the mPAP and the ratio of the Cav-1 expression within medial:intimal layer of the vessels ($r2 = 0.56$, $p = 0.003$).
Conclusion: Here we show for the first time that the expression of Cav-1 within pulmonary arteries is associated with pulmonary hypertension in COPD-patients. Since Cav-1 has been found to play an important role in the production of endothelial NO and, moreover, appears to regulate fibrosis a least in part, our results emphasize a potential novel player in the pathogenesis of COPD-associated pulmonary hypertension.

P123

Use of B-type natriuretic peptides in the diagnosis of unexplained exercise intolerance

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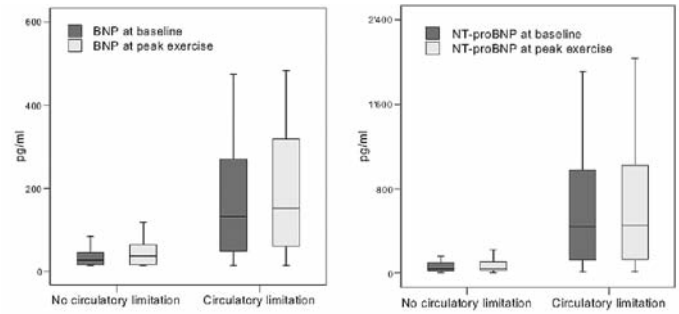
Background: Exercise limitation is common and difficult to evaluate. Cardiopulmonary exercise testing (CPET) is the method of choice for the differentiation of exercise intolerance. However, CPET is costly and not widely available. We evaluated the use of B-type natriuretic peptide (BNP) and amino-terminal pro-brain natriuretic peptide (NT-proBNP), both quantitative markers of cardiac stress, in the identification of circulatory limitation.
Methods and results: A total of 163 consecutive patients with unexplained exercise intolerance underwent CPET. Levels of BNP and NT-proBNP were determined before and one minute after maximal exercise. In 107 patients the cause of exercise limitation could be determined with at least 70% certainty using all data pertaining to the individual patient including CPET findings. In 37 of these patients (35%), circulatory limitation was identified. BNP and NT-proBNP levels at rest (median 132 and 441 versus 27 and 42 pg/ml) and at peak exercise (median 152 and 455 vs. 37 and 44 pg/ml; p <0.001 for all comparisons) were significantly higher in patients with circulatory limitation as compared to those with other limitations. The areas under the receiver operating characteristic curve for the ability of BNP and NT-proBNP to identify circulatory limitation were 0.81 (95% confidence interval 0.72–0.91) and 0.85 (95% confidence interval 0.76–0.93) for baseline, and 0.79 (95% confidence interval 0.69–0.89) and 0.84 (95% confidence interval 0.76-0.92) for peak exercise levels (all p <0.001).
Figure: Baseline and peak exercise B-type natriuretic peptide (BNP) (left) and amino-terminal pro-brain natriuretic peptide (NT-proBNP) (right) levels in patients with and without circulatory limitation of exercise.
Conclusions: BNP and NT-proBNP have high accuracy in the identification of circulatory limitation and might be valuable simple and inexpensive diagnostic markers in the evaluation of exercise intolerance.



P124

Pulmonary rehabilitation in Switzerland

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Introduction: In 2002, the ALVR criteria (Arbeitsgemeinschaft Leistungserbringer und Versicherer in der Rehabilitation) on pulmonary rehabilitation (PR) were published (1). Since January 2005, Swiss health insurance companies are obliged to pay for PR (2), whereas no fixed tariff was agreed for out-patient programs. Our WG defined quality criteria for PR programs. For accreditation of a specific program, audits must be performed by two specialists. The audit focuses on the quality of structure and the rehabilitation process, as well as outcome measurements. Currently 11 in- and 49 outpatient programs are accredited.
Methods: To assure the quality of programs, assessment is done every two years by questionnaire, with assessment of numbers of patients treated, their diagnoses and complications. 10 inpatient and 36 outpatient programs returned the questionnaire. Failure to reply to two consecutive questionnaires results in the loss of accreditation.
Results: In 2005 and 2006 respectively 4103/4279 patients were treated in an in-patient and 3682/3743 in an out-patient setting. 77% of the program directors answered the questionnaire. Although mainly COPD patients (41.3% 2005, 41,0% 2006) are enrolled, also patients with other diagnoses qualifying for PR (for listing cf.1). Acute exacerbations occurred in 74, fatal complications in 4 cases. Over 95% of the patients completed the program.
Conclusions: Switzerland has a network of PR programs in out- and inpatient settings. The programs are well organised and meet international quality criteria. The biennial questionnaire is an excellent tool to maintain a high program quality.
We are convinced that a too small proportion of COPD patients in Switzerland is enrolled in PR programs. GPs, pulomonologists and hospitals should be better informed to enrol more patients in PR programs.
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P125

Kinetics of haemoglobin oxygen saturation measured by continuous pulse oximetry during the 6-minute walking test

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Background: The distance walked during a 6 minute walking test (6MWT) is commonly used to assess functional exercise capacity in cardiopulmonary diseases. Previous studies suggest that measuring exercise haemoglobin oxygen saturation by pulse oximetry (SpO2) may be of prognostic value in certain diseases. However, no consensus exists on the role and the methods of pulse oximetry to detect exercise induced hypoxemia during the 6MWT. The aim of this study was to analyze the kinetics of SpO2 during the 6MWT by using continuous pulse oximetry.
Methods: We studied 106 patients (age 53.5±18.1 yrs, 51 female) with various restrictive, obstructive, or vascular lung diseases. The 6MWT was performed following strictly the American Thoracic Society guidelines. In addition, SpO2 was continuously measured and then downloaded and analyzed (Nonin 4000 Avant). Values considered were: before the test (SpO2pre), lowest (SpO2min), and at the end of the test (SpO2end). Exercise desaturation was defined as a fall of ≥4% of SpO2.
Results: 94 patients completed the 6MWT and 12 stopped before 6 min. Whereas SpO2end corresponded to SpO2min in 26 patients, SpO2min occurred before the end of the test in 80 patients. Exercise desaturation was absent in 19 patients: SpO2pre was 94.7 ± 3.2%, SpO2min was 93.1 ± 3.1%, and SpO2end was 94.5 ± 3.7%. Exercise desaturation was present in 87 patients: SpO2pre was 93.7 ± 3.8%, SpO2min was 83.4 ± 6.8%, and SpO2end was 85.8 ± 7.1%, meaning that desaturation was 30% deeper if SpO2min was considered instead of SpO2end. In this group, 17/87 patients (20%) would not be considered as showing exercise desaturation if only SpO2end was recorded.
Conclusion: Maximal desaturation occurs before the end of the 6MWT in a majority of patients. Thus, considering a single value at the end of the test may be misleading. Continuous pulse oximetry during the 6MWT yields a more complete picture of exercise induced hypoxemia and may be of help in the assessment and follow-up of patients.

P126

Lenalonamid induced interstitial pneumonitis

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We report the case of a 66 years old patient known for a stage IIIa multiple myeloma since 2006 previuosly treated with VAD chemotherapy, Thalidomid and two autologous stem cell transplantation. Recently a Lenalonamid (Revlimid) therapy was started without concomitant dexamethason. He begun to feel subfebrile temperatures, increasing cough and fatigue without marked dyspnea. Laboratory analyses showed increased inflammatory parameters with a CRP of 137, a Procalcitonin level of <0.05 and a marked eosinophilia (20%). Conventional chest x-ray showed only very mild interstitial thickening. Pulmonary function test showed a mild restriction with moderate non-reversible obstruction and severe impairment of difussion capacity. A PFT of one year before was absolutely normal and the patient never had pulmonary diseases.
A high resolution CT-scan detected bilateral ground glass opacities, localised mainly in the upper lobes. Bronchoscopy was performed and an infectious cause was ruled out. BAL showed a mixed pattern with an increase of neutrophils (41%), lymphocytes (15%) and eosinophils (6%). The CD4/CD8 quotient was slightly decreased to

1.6. Transbronchial biopsies showed focal interstitial pneumonitis with granulomatous aspects compatible with a toxic-drug induced damage.
Lenalonamid was stopped and steroid introduced (50 mg a day). After three weeks chest x-ray returned to normal, PFT values normalised, fever, cough and fatigue disappeared and CRP and eosinophils levels normalised.
Conclusion: To our knowledge this is the first described case of Lenalonamid induced interstitial pneumonitis. This side effect may be underestimated because of frequent concomitant use of dexamethason while starting with Lenalonamid.

P127

Diagnostic yield of real-time EBUS TBNA in conventional bronchoscopic TBNA negative mediastinal and hilar lymphadenopathy

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Real time endobronchial ultrasound (EBUS) TBNA has been shown to have a higher diagnostic yield for the staging of lung cancer in patients with mediastinal lymphadenopathy compared to conventional TBNA except for infracarinal lymphnodes. However, the EBUS bronchoscope is not very suitable for the diagnosis of endobronchial lesions or peripheral nodules. There are two possibilities to overcome this problem. Either to start with conventional bronchoscopy to diagnose the peripheral lesion and than to change to EBUS for mediastinal staging (or vice versa) or to perform mediastinal staging with conventional TBNA followed by brush/TBNA/TBB for the diagnosis of the peripheral lesion using the same bronchoscope. If conventional TBNA is negative EBUS is than performed as a second procedure. In this study we analysed the diagnostic yield of EBUS TBNA in patients in whom conventional TBNA of mediastinal lymphnodes was negative or in whom assessment of hilar lymphnodes needed ultrasound localization. There were 50 patients (39 male; 11 female) with a mean age of 66.2 years. EBUS TBNA was performed under local anesthesia and combined sedation with hydrocodone and propofol. The following lymph nodes were sampled: 28 paratracheal right; 5 paratracheal left; 17 subcarinal; 12 hilar right; 3 hilar left. Overall, there were 23/60 (38%) positive and 15/60 (25%) true negative lymph nodes at EBUS TBNA. Therefore, the overall accuracy was 63%. 19/45 (42%) mediastinal lymph nodes were positive at EBUS TBNA and 11/45 (24%) were true negative. Therefore, the accuracy was 66%. 4/15 (27%) hilar lymph nodes were malignant at EBUS and 4/15 (27%) were true negative. Therefore, the accuracy was 54%. Three lymph nodes were false negative at EBUS TBNA (PET positive) and were confirmed to harbor malignancy at surgical evaluation. Conclusion: Conventional TBNA and EBUS TBNA can be used as complementary tools in a step wise fashion. EBUS TBNA increases the positive diagnostic yield in mediastinal lymphadenopathy if conventional TBNA is inconclusive and often allows to get a diagnosis if hilar lymph nodes are enlarged. The integration of conventional TBNA with CT/PET and eventually EBUS TBNA allows to properly assess mediastinal and hilar lymphadenopathy in suspected or proven lung cancer.

P128

Aetiology of bronchoscopic TBNA negative PET positive non-malignant lung nodules

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Positron emission tomography (PET) has been well established as a method for mediastinal staging of lung cancer and for detection of distant metastases (Lardinois et al N Engl J Med 2003;348:2500–7). A combination of bronchoscopic TBNA revealing no malignant cells with a negative PET has a high negative predictive value for non-malignant mediastinal lymphadenopathy (Bernasconi et al Eur Respir J 2006;27:889–894). Similarly the negative predictive value of TBNA combined with PET is good in peripheral lung nodules larger than 1 cm in diameter (Chhajed et al Chest 2005;128:3558-3564). If PET is positive and bronchoscopic TBNA negative further evaluation with invasive methods is needed. However not all PET positive lung nodules and infiltrates are of malignant origin. We analysed the aetiology of 21 PET positive but bronchoscopic TBNA negative lesions which turned out to be benign on histology. There were 11 male and 10 female patients with a mean age of 61.1 years. Final diagnostic procedures to achieve a definitive histological diagnosis consisted of video-assisted thoracoscopic surgery (8; two of them combined with LVRS), lobectomy (4), open lung biopsy (2), transbronchial biopsy (5) and CT guided biopsy (2). Final non-malignant diagnoses revealed cryptogenic organizing pneumonia (6), aspergilloma (2), granuloma (2), tuberculosis (1), atypical mycobacteriosis (1), M. Wegener (1), lung sequester (1), sarcoidosis (1), eosinophilic pneumonia (1), fibrosing alveolitis (1), rheumatic nodule (1) and unspecific inflammatory changes (3).
Summary and conclusion: despite a very good sensitivity of the combination of PET with bronchoscopic TBNA for peripheral lung nodules larger than 1 cm in diameter the specificity of PET is limited. False positive PET results can be caused by different types of infection but not infrequently also by cryptogenic organizing pneumonitis or other noninfectiuos inflammatory diseases. In cases or PET positive peripheral infiltrates or nodules and cytologically negative bronchial washings, brushings and/or TBNA histological confirmation of malignancy should be obtained by TBB, CT guided biopsy or VATS prior to decide about the extent of pulmonary resection.

P129

Clinical practicability of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA)

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Aim: To investigate clinical practicability and accuracy of EBUS-TBNA with Olympus EBUS-Bronchoscope (BF-UC 160F) for staging or diagnosis of mediastinal and hilar lymph nodes (LN) in patients with known or suspected malignancy.
Methods: Prospective evaluation of performed EBUS-TBNA since 3/07. The following parameters were recorded: Duration of entire intervention and EBUS-TBNA, needed manpower, complications, cytological results and accuracy. The investigation was always started with conventional videobronchoscopy followed by EBUS-TBNA during the same session. On-site cytological evaluation was always available.
Results: Between 3/07 and 12/07 42 EBUS-TBNA were performed in 39 patients (3 investigated twice). In 29 (73%) EBUS-TBNA was the only endoscopic investigation. 41 (98%) interventions were done under topical anesthesia and conscious sedation (Hydrocodon and mostly Midazolam). In general one investigator (TH or MH) and two nurses performed EBUS-TBNA. Mean duration of whole investigation was 58min (95% CI 51–64) and of EBUS-TBNA 43 min (36–49). On average 2 (range 1–4) LN were evaluated with 3.6 (3.3–3.9) TBNA per LN (total 71 punctured LN). Mean short and long axis diameter of LN on CT were 12 mm (10–14 mm, 27<10 mm) and 17 mm (15–20 mm, 7<10 mm), respectively. 21(30%) of LN showed malignancy, 48 (68%) lymphocytes and only 2 TBNA were not diagnostic. One patient suffered from acute laryngospasm, one from severe coughing and one procedure was not possible due to insufficient sedation and was repeated under general anesthesia. In 16 patients with lung cancer and suspected N2/3 disease on CT EBUS-TBNA confirmed N2/3 stage in 6, one with negative PET/CT. N2/3 disease was excluded in 10, EBUS-TBNA showing only lymphocytes without malignancy confirmed by extensive surgical nodal stating. In 5 patients a nodal metastase of a non lung carcinoma was found and 2 had metastatic lung carcinoma. The remaining 16 patients with mediastinal or hilar lymphadenopathy without suspicion of malignancy showed only lymphocytes or granulomatous inflammation in the EBUS-TBNA with no carcinoma during follow-up in 15, mediastinoscopy is planned in one.
Conclusion: EBUS-TBNA can routinely and safely be performed in an ambulatory setting under topical anesthesia and conscious sedation with good time-performance requesting reasonable manpower. EBUS-TBNA is an accurate tool for mediastinal/hilar nodal staging or diagnosis.

P130

Molecular classification of non-small-cell lung cancer and prediction of survival using gene expression signatures derived from clinical routine biopsies

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Background: The usefulness of microarray-based technologies for the classification and prediction of outcome of non-small-cell lung cancer (NSCLC) has been shown previously. However, the need for large tumor samples limits its application to operated early stage tumors. The aim of this study was to evaluate the feasibility and utility of gene expression profiling using biopsies obtained during routine clinical work-up including minute bronchoscopic biopsies.
Methods: Gene expression profiles (Novachip; 34207 transcripts) derived from biopsies of 41 consecutive chemotherapy-naïve NSCLC patients (mean follow-up: 22 months) and 15 controls with inflammatory lung diseases were analyzed. Gene signatures associated with different NSCLC phenotypes and survival were identified and confirmed using independent NSCLC data sets and tissue microarrays.
Results: Classification based on functional genomics showed an overall sensitivity and specificity of 80% and 89% respectively, and was dependent on the proportion of tumor cells present in the biopsies. From an optimized metagene of 44 prognostic genes, 13 could be validated in 4 independent data sets on lung cancer. A metagene based on these 13 genes was further validated in an independent data set. The prognostic value of VEGFB expression was confirmed on a protein level by tissue microarray.
Conclusion: The proposed strategy allows molecular tumor classification and prediction of survival in patients with NSCLC of all stages and is suitable for an integration in the daily clinical practice.

P131

Comparing right lower lobe sleeve lobectomy with bilobectomy in the treatment of bronchial carcinoma

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Introduction: A tumour located in the right lower lobe, especially when arising in segment 6 has a close anatomical relationship to the middle lobe bronchus, which may make lower bilobectomy necessary. This procedure has an increased morbidity and mortality when compared to lobectomy. In this situation, sleeve lobectomy may be an alternative. These procedures are compared in terms of postoperative lung function, complication rate and oncological radicality.
Patients and methods: Retrospective analysis of 52 patient records between 01.01.2005 and 31.12.2007 operated on an NSCLC (stage I-IIIb)of the right lower lobe. In 28 patients, a sleeve lobectomy with preservation of the middle lobe was performed, in 24 patients a bilobectomy. The average age was 63 years. Data recorded was: TNM classification, preoperative lung function and after at least 3 months, length of chest tube drainage in days, complications and rate of recurrence.
Results: Preoperative FEV_1 was comparable in both groups (sleeve lobectomy 74%, bilobectomy 76%). After sleeve lobectomy we observed one early anastomotic leakage that was due to a technical failure, requiring reintervention. No patient died. After bilobectomy no bronchial leakage was observed, one patient died of apoplexy during the hospital stay. Radical resection (R0) was achieved in 50/52 patients. The length of the operation was 121 min. for sleeve lobectomy and 144 for bilobectomy. Chest tube were removed on average in both groups after 5 days. After bilobectomy postoperative FEV_1 was on average 69%, whereas after sleeve lobectomy it was 78%.
Conclusions: Preservation of the middle lobe by means of a sleeve lobectomy is possible in almost half the patients. We had no evidence that this method was less radical. The complication rate was similar in both groups. The complete analysis of postoperative lung function and rate of recurrence will be presented.

P132

Diagnostic value and risk assessment of surgical lung biopsy in patients with pulmonary complications after high-dose chemotherapy and stem cell transplantation

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Pulmonary complications are frequent in patients with hematologic malignancies. Due to the variety of infectious and non-infectious pulmonary complications non-invasive and invasive diagnostic methods are used to initiate adequate treatment. Fears to perform an invasive surgical approach include bleeding, infection and respiratory failure. We therefore analysed the diagnostic value and postoperative complications of VATS or open lung biopsy in 53 patients with hematologic malignancies undergoing lung surgery between 2000 and 2007. 16 patients were treated with high dose chemotherapy and 37 patients with allogeneic or autologous stem cell transplantation. VATS biopsy was performed 37, OLB in 16 and diagnostic as well as therapeutic lung resection in 17 cases. 18.9% of patients were severely neutropenic (<500 neutrophils) and 34.0% of patients markedly thrombopenic (platelets <50'000) at the time of surgery. An infectious cause of the pulmonary complication was documented in 19 (35.8%) of patients whereas a non-infectious pulmonary complication was present in 34 (64.2%) of patients. Histological assessment revealed invasive fungal infection in 14 (26.4%), bronchiolitis obliterans in 16 (30.2%), acute interstitial pneumonitis in 11 (20.8%), diffuse alveolar damage in 4 (7.5%) and bacterial infection in 3 (5.7%) patients respectively. Mycobacterial infection was demonstrated in one patient and an inconclusive result was obtained in another patient. Surgical biopsy resulted in a change of therapy in 85.0% of cases. Respiratory failure developed in 4 patients and ventilation was already needed prior to biopsy in two patients. Intra- or postoperative bleeding could easily be managed in all patients whereas wound infection occurred in one patient. 30-day-mortality was 9.4%. These patients died because of respiratory failure (3 patients with diffuse alveolar damage), multi-organ failure (1) and myocardial infarction (1). A prolonged postoperative ICU stay (>72 hours) was needed in 18.9% of patients. Patient survival was mainly influenced by the type of the pulmonary complication and underlying hematologic malignancy and not by the surgical procedure.
Summary: surgical lung biopsy has a high diagnostic yield and is associated with acceptable morbidity and mortality in patients with pulmonary complications following high dose chemotherapy and stem cell transplantation in whom non-invasive methods and bronchoscopy fail to reveal a definitive diagnosis.

P133

Medical thoracoscopy: morbidity and mortality in a series of 534 patients

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In the Department of Pulmonology 534 thoracoscopies were performed between 1/1991 – 9/2007, 385 of which were for the purpose of talc pleurodesis, 149 were for diagnostic reasons. T.were carried out in the endoscopy suite under local anaesthesia and sedation with opioids and Midazolam. In this retrospective series we have evaluated morbidity and mortality without considering pain during or after the procedure. 8 patients had substantial side effects, two of them died. 1 patient with ovarian cancer developed pulmonary embolism during thoracoscopy and died 6 hours later. 1 pat. with breast cancer developed PE 10 days after the procedure and causality with the pleurodesis could be discussed. 2 patients had bradycardia and hypotension and needed vasoactive treatment; the procedures had to be stopped. 2 patients had bleeding after cutting adhesions with electrocautery and after biopsy. There was no need for transfusion and bleeding could be stopped by compression of the bleeding site. 1 pat. had a localized infection at the chest tube entrance and had to be treated with antibiotics. The eighth patient developed atrial fibrillation and cerebrovascular infarction 2 days after T. Morbidity of medical thoracoscopy in our series was 1.50%, mortality 0.37% and it is questionable, if death because of pulmonary embolism in patients with a progressive malignant disease was due to the procedure. Since we have treated patients routinely with heparin after thoracoscopy, we have not had any more thrombembolic complications. Bleeding is a rare complication and may be avoided by choosing the biopsy site «over the rib». Infection was rare in our series and no pat. developed empyema. Surgical intervention was never necessary. Cardiovascular problems may be a problem. In patients with known severe cardiopathy, we prefer pleurodesis with talc slurry, which is less invasive, than thoracoscopy. In conclusion medical thoracoscopy is a safe procedure with a low morbidity (1.50%) and mortality (0.37%). Prevention of thrombembolism is recommended. Bleeding after Biopsy and infections are rare. The great advantage of medical thoracoscopy is the low cost in comparison with VATS.

P134

Hughes-Stovin syndrome presenting with life-threatening haemoptysis: a case report

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Background: Hughes-Stovin syndrome is an extremely rare pathologic entity of unknown origin presenting with pulmonary artery mural thrombi and aneurysms and venous thrombosis (1). Some studies have suggested that Hughes-Stovin syndrome may represent an atypical manifestation of Behçet's disease (2).
Methods: We herein report the case of a 17-year old male who initially complained of recurrent hematemesis and hemoptysis in the past 6 months. In September 2006, he was admitted on ICU and intubated because of severe hemoptysis with acute respiratory failure and anemia (Hb 66g/L).
Results: Initial diagnostic work-up revealed the presence of thrombosed pulmonary artery aneurysms of the left upper and lower lobes. Common causes of pulmonary artery aneurysms without arterio-venous communication (congenital vascular and cardiac abnormalities, pulmonary hypertension, mycotic and infectious disease including tuberculosis, vasculitis and other systemic diseases) were excluded. Major diagnostic criteria of the Behçet's disease were not fulfilled. Though deep venous thrombosis at typical localization (lower extremities) was absent in this case, the presence of filling defects of the upper pulmonary vein at pulmonary CT angiogram as well as the absence of another likely differential supported the diagnosis of Hughes-Stovin syndrome. Our patient was initiated on immunosuppressive treatment associating prednisolone with monthly pulses of cyclophosphamide, subsequently substituted by oral azathioprine. Frequent clinical follow-ups and sequential CT angiogram studies revealed a complete regression of both pulmonary aneurysms. Moreover, the patient remained in excellent health and has not reported additional hemoptysis since.
Conclusions: This case exemplifies that pulmonary aneurysms associated to Hughes-Stovin Syndrome respond to immunosuppression and should not receive anticoagulation despite presence of thrombosis.
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P135

An unusual cause of haemoptysis: new diagnosis of an anomalous origin of the left pulmonary artery from the ascending aorta in a 20-year old man

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A 20-year-old man with known 22q11 microdeletion and DiGeorge syndrome was admitted because of hemoptysis.
DiGeorge Syndrome was diagnosed six years ago with detection of a right aortic arch, a PDA to the right pulmonary artery and an aberrant left subclavian artery. Subsequent analyses disclosed a microdeletion at chromosome 22q11.2.
At the most recent admission, chest CT showed an until now not noticed anomalous origin of the left pulmonary artery (LPA) from the ascending aorta. At cardiac catherization, a non-stenosed LPA originating from the aorta and systemic pressure in the left lung were documented. The right ventricle was connected to the single right PA. Right mean PA pressure was mildly elevated at 30 mmHg. We interpreted the hemoptysis as a consequence of pulmonary hypertension in the left lung and suspected superinfection as cofactor. Hemoptysis stopped spontaneously.
There is irreversible pulmonary vasculopathy in his left lung due the anomalous origin of the LPA. Fortunately, the decrease in left lung blood flow over time due to the progressive increase in pulmonary artery resistance reduced the volume load on the left ventricle. The PDA shunt flow is small and does not require intervention.
Concerning the mild pulmonary hypertension in the RPA, we abstained from bosentan therapy because of mild symptoms and its potential to enhance LPA blood flow and thereby left ventricular volume load.

P136

Infliximab therapy in patients with chronic progressive sarcoidosis – a retrospective follow-up study

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Background: Infliximab is a monoclonal antibody against tumor necrosis factor-alpha, and its efficacy has been evaluated in patients with chronic sarcoidosis, demonstrating a significant improvement in percentage predicted forced vital capacity (FVC, %P) at week 24 (1). However, the clinical importance and the long-term benefit of infliximab treatment remain unclear.
Methods: A retrospective chart review of all patients with histologically-confirmed pulmonary and/or extrapulmonary sarcoidosis who received infliximab (typically 3 mg/kg, 4-/6-/8-weekly intervals) for chronic steroid-resistant disease between January 2003 and November 2007 at the Clinic of Pneumology, University Hospital Basel. Lung function, 6-minute walk distance, cardio-pulmonary exercise tests, chest x-rays, index lesions (e.g. extent of skin involvement), and quality of life questionnaires, before and on/after infliximab therapy were assessed. Sarcoidosis is a heterogeneous disease, and responses to treatment are heterogeneous too; outcome measures were, therefore, defined as individualized treatment targets which were assessed for each subject individually.
Results: In total 27 patients received infliximab, 11 (41%) patients with predominantly pulmonary involvement, and 16 (59%) patients with predominantly extrapulmonary disease (8 CNS, 5 lupus pernio, 1 eye, 1 hypercalciuria, and 1 heart). The mean duration of treatment was 14 (range 2 to 59) months. In 2/27 (7%) patients the treatment had to be stopped due to adverse effects (1 liver enzyme elevation, 1 allergic reaction). There was one drop-out after a single dose of infliximab due to missing cost coverage. Two of 11 patients (18%) with pulmonary sarcoidosis showed a >10%-improvement of FVC,%P, 7 (64%) patients showed a 0-10%-improvement, and in 2 (18%) patients FVC,%P declined during infliximab treatment. Of the 15 evaluable patients with mainly extrapulmonary sarcoidosis 6 (40%), 6 (40%) and 3 (20%) showed a complete remission, partial remission, and no response, respectively. Thus, overall 21/26 (81%) patients profited from infliximab treatment.
Conclusions: Preliminary analysis of this retrospective follow-up study indicates that infliximab is very efficient in patients with steroid-resistant sarcoidosis when assessed with individualized treatment targets. Patients with predominantly extrapulmonary sarcoidosis seem to profit more than patients with predominantly pulmonary disease.
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P137

Chloroquine-induced acute eosinophilic pneumonia – a case report and review of the literature

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Introduction: Chloroquine has a long-standing tradition in prophylaxis and treatment of malaria tropica, rheumatoid and autoimmune disorders including sarcoidosis. Common side-effects are frequently related to the gastrointestinal tract and central-nervous-system, but pulmonary unwanted secondary effects are rarely reported.
Methods: We herein report the case of a previously healthy 41-year old woman, who presented with dry cough, cyanosis, and acute hypoxic respiratory failure, 13 days following chloroquine administration for rosacea.
Results: Admission laboratory examinations showed increased inflammatory markers without blood eosinophilia. Initial Chest X-ray revealed Kerley-B-lines and the CT-thorax showed discrete subpleural ground-glass opacities. In pulmonary function test, a moderate obstruction was observed (FEV_1 62%). The BALF examination demonstrated eosinophilia (17%) combined with mildly elevated neutrophils (11.5%), and histopathology of transbronchial biopsies showed eosinophilic granulocytes in the interstitial lung tissue. No evidence for an infection, including bacterial, viral, helminthic and fungal pathogens was found. Prick-test for Aspergillus fumigatus was not in favour of an allergic broncho-pulmonary aspergillosis. Other causes of elevated BALF eosinophilia, such as Churg-Strauss-syndrome, HIV-infection, and hypereosiniphilic-syndrom / chronic eosinophilic leukaemia were excluded. The time-course of events, as well as the radiological and BALF finding were not characteristic for an idiopathic acute eosinophilic pneumonia. The symptoms subsided within 3 days following the cessation of chloroquine. In the literature, only 2 further well documented cases are reported with similar time-courses and findings. Typically, the reported latency was 1 to 2 weeks and the symptoms subsided within a few days after discontinuing chloroquine. In all cases BALF

eosinophilia was noted. All patients were active smokers and developed a blood eosinophilia with a latency of 4 to 6 days. In 2 cases elevated IgE-levels were measured.
Conclusion: Though pulmonary side-effects seem to be rather uncommon, the possibility of chloroquine-induced pneumonitis should be considered if a suggestive clinical course exists following exposure to this substance.

P138

Sleep related breathing disorders in patients with pulmonary hypertension

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Background: Cheyne-Stokes respiration and central sleep apnea (CSRA/CSA) are common in patients with left heart failure. We investigated the hypothesis that sleep disordered breathing is also prevalent in patients with right ventricular dysfunction due to pulmonary hypertension.
Methods: We studied 38 outpatients (mean ± SD age 59 ± 16 years, 11 males) with pulmonary arterial hypertension (n = 23) or thromboembolic pulmonary hypertension (n = 15). NYHA class was 2-4, the 6 minute walk distance was 481 ± 120 m. In-laboratory polysomnography (n = 22) and ambulatory cardio-respiratory sleep studies (n = 38) including pulse oximetry were performed. Quality of life and sleepiness by Epworth score were assessed.
Results: The apnea/hypopnea index was 14±14 events/h with 10 ± 9 central and 3 ± 10 obstructive events/h. Seventeen patients (45%) had ≥10 apnea/hypopnea events/h. Comparison of 13 patients with ≥10 CSR/CSA events/h with 21 patients with <10 CSR/CSA events/h (excluding 4 patients with ≥10 obstructive events/h from this analysis) revealed no difference in regard to hemodynamics, NYHA class and Epworth sleepiness scores. However patients with ≥10 CSR/CSA events/h had a reduced quality of life in the physical domains. Ambulatory cardio-respiratory sleep studies accurately predicted ≥10 apnea/hypopnea/h during polysomnography in patients who underwent both studies (area under the receiver operating characteristic curve 0.93 ± 0.06 p = 0.002). The corresponding value for pulse oximetry was 0.63 ± 0.14 (p = ns)
Conclusions: In patients with pulmonary hypertension CSR/CSA is common but obstructive sleep apnea also occurs. Sleep related breathing disorders are not associated with excessive sleepiness but affect quality of life. They should be evaluated by polysomnography or cardio-respiratory sleep studies since pulse oximetry may fail to detect significant sleep apnea.

P139

Is long-term treatment of obstructive sleep apnoea syndrome with autoCPAP equivalent to fixed CPAP?

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Introduction: CPAP therapy of the obstructive sleep apnea syndrome (OSA) with continuous computer controlled mask pressure adjustment (autoCPAP) has potential advantages over treatment with fixed pressure (fixedCPAP). Whether autoCPAP is equivalent to fixedCPAP during long-term treatment is uncertain. Therefore, we initiated a multi-center trial to compare the two treatment modes in OSA patients during 2 years. We report results of an interim analysis.
Methods: Consecutive patients with OSA (apnea/hypopnea index AHI >10/h, Epworth score >8) were randomized to autoCPAP or fixedCPAP therapy at the 90.%-ile of mask pressure during a 2–4 weeks autoCPAP adaptation period. At baseline and at 3 months we assessed sleepiness, quality of life, AHI, and 24h blood pressure. Equivalence of CPAP modes was evaluated by computing 95% confidence intervals of differences in treatment effects.

Table. Treatment effects of autoCPAP and fixedCPAP

	Change at 3 months		Difference between treatment effects (changes) autoCPAP - fixed CPAP	
	AutoCPAP n=33	FixedCPAP n=22	Mean value (#)	95% confidence interval
Epworth score	-5.3±4.6*	-4.5±3.8*	-0.9 (-)	-3.2 to 1.5
SF-6D utility	0.04±0.1*	0.01±0.13	0.03 (+)	-0.03 to 0.09
SF-36 vitality	17±21*	21±19*	4 (+)	-15 to 7
AHI (1/h)	-46.6±24.7*	-48.3±19.7*	1.6 (-)	-10.8 to 14.1
OSLER sleep resistance time (min)	5.8±10.6*	7.6±11.6*	-1.8 (+)	-7.8 to 4.3
CPAP use	5.1±2.1	5.7±2.1	0.6 (+)	-0.5 to 1.7

* P<0.05 for change vs. baseline; # the +/- sign indicates whether positive or negative differences indicate superiority of autoCPAP.

Results: 74 patients were recruited, 55 were followed-up for >3 months. Their mean ± SD age was 55 ± 12 y. Data on patients with complete follow-up at 3 months are presented in the table.
Conclusions: AutoCPAP and fixedCPAP both provided major improvement in main clinical outcomes of OSA patients. None of the two modes was superior to the other. Improvement in subjective sleepiness can be considered equivalent.
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P140

The Velumount® device to treat obstructive sleep apnoea: does it work?

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Introduction: The Velumount is a non approved device developed to reduce snoring and obstructive sleep apnoea (OSA). It is applied intraorally during night and is targeted to remove the soft palate from the dorsal pharnygeal wall. Despite an almost total lack of published medical data, due to an intensive media campaign, this device is widely used in Switzerland.
Methods: I collected data from all sleep apnoea patients of our clinic, known to have tried the Velumount. I asked for general acceptance of the device, side effects, reduction of snoring and daytime sleepiness. In addition, nocturnal oximetries were performed and the results were compared with the baseline data.
Results: 42 patients of our clinic tried the Velumount device. Information was available from 39 patients. All 39 patients were diagnosed with OSA syndrome (AHI 26 ± 11 / h, desaturation index (DI) = 23 ± 12 / h, Epworth Sleepiness Score (ESS) = 12.2 ± 4.2 points). 19 patients were not able to tolerate CPAP, 20 patients used CPAP but wanted to try an alternative treatment.
Compared with baseline, at the time of data acquisition under Velumount treatment, these 39 patients had lost 3.1 ± 3.0 kg of weight. 17 of these 39 patients (44 %) were not able to tolerate the Velumount. 22 patients were able to use the Velumount device at least 4 hours per night. In these 22 patients the ESS fell from 12.5 ± 5.3 to 9.6 ± 4.7 points (p = 0.02). All patients but one reported a clear reduction in snoring. In 15 of these 22 patients a nocturnal oximetry under velomount treatment was performed: the DI fell from 22 ± 11 at baseline to 16 ± 10 / h (p = 0.04). However, in 12 of these 15 patients the DI under CPAP was 4 ± 4 / h.
Conclusions:
1. Approximately 40% of patients do not cope with the Velumount.
2. Those who manage to use the Velumount feel a significant and relevant reduction of sleepiness and a clear reduction in snoring.
3. Though statistically significant, the Velumount does not improve overnight oximetry results relevantly.
4. The changes from baseline described above could be in part due to a weight loss of approx. 3 kg.
5. Considering the discrepancy between subjective improvement in sleepiness and only small changes in oximetry, it seems likely, that some of the beneficial effects of the Velumount are due to a placebo effect.
6. In conclusion, these scarce data do not support the use of the Velumount as a sufficient alternative therapy to CPAP.

P141

Spirometries in patients at a high risk of developing COPD

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Introduction: Chronic obstructive pulmonary disease is a leading cause of mortality and morbidity worldwide and can be easily detected in its preclinical phase by office spirometry.
Aims: To investigate the effectiveness of spirometry for casefinding in smokers at risk of developing chronic obstructive pulmonary disease who visit their general practitioner.
Methods: A prospective study of smokers aged 40 and above visiting their general practitioner with a short interviewer administered questionnaire (about respiratory symptoms, reason for consultation) and office spirometry.
Results: 8301 smokers underwent office spirometry. Mean age was 54 years (range 40–90 years). 57% of participants were male. According to the modified Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria by Hardie et al. 893 (11%) were having mild, 1050 (13%) moderate, 360 (4%) severe and 86 (1%) very severe obstruction. 4781 (58%) of the participants were complaining respiratory symptoms: 3432 (41%) were complaining of cough, 2284 (28%) phlegm and 3288 (40%) dyspnea. Having respiratory symptoms did not predict presence or severity of obstruction. The reason for the consultation were problems regarding musculoskeletal system in 1314 (16%), cardiovascular system in 1388 (17%), respiratory systemin 1535 (19%), gastrointestinal system in 345 (4%) and others in 3719 (45%).
Conclusions: 29% of smokers aged 40 and above in a general practice sample in Switzerland have chronic obstructive pulmonary disease. A significant proportion of these smokers do not complain of respiratory symptoms.

P142

A structured, intensive smoking cessation programme: rauchSTOPP Basel

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Background: It is well known that a structured program improves the chance of successful smoking cessation. We therefore designed and initiated a structured smoking cessation program at the three largest employers of the region of Basel, the University Hospital Basel (USB), F. Hoffmann- La Roche Ltd. and Novartis Pharma AG. The program was offered to all employees.
Methods: A total of 10 visits (8 personal attendances, 2 telephone visits) are performed over a period of 24 months (at day 1, at week 1, 2, 4, 6, 12, at month 6, 12, 18 and 24). An internet based system is used for schedule of visits, data documentation and data analysis. Specifically trained nurses and doctors consult smokers. Fagerstroem and quality of life questionnaires were used. The success of smoking cessation was measured by exhaled CO. Nicotine replacement (patch, gum, inhaler) combined with bupropion was offered to all subjects without contraindications. The smoke stop day is scheduled for day 7. Spirometry was measured at inclusion, at 12 and 24 months. For smokers reporting relapse, a re-intervention was offered.
Results: Between March 2005 and March 2006 a total of 887 smokers have been included in the program. A total of 6293 visits and 328 re-intervention visits were performed so far. Bupropion was used in 491 patients (55.9%), a combination of two nicotine-replacement therapy in 455 patients (51.76%) and one nicotine-replacement therapy in 292 patients (33.22%). Carbon-monoxide proven point abstinence-rates were 67.3% at 4 weeks, 50.5% at 12 weeks, and 34.0% at 12 months, respectively.
Conclusion: A large structured interventional smoking cessation program in Switzerland is feasible and well perceived by the employees. Abstinence rates at one-year exceed the results of most published clinical trials. Intervention nurses and doctors have to be specifically trained. The time and resources needed for such an intervention program should not be underestimated.

P143

Teaching smoking cessation for medical students: evaluation of the effect on knowledge, skills and attitude

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Background/Aims: Smoking is the most harmful consume behaviour of our society. Medical education is usually theory-based and only few pragmatic contents are found in curricula. This may lead to deficits in motivation and counselling of smokers. We therefore performed and evaluated a smoking cessation counselling course.
Methods: 88 medical students (53 f, 35 m) were educated by a doctoral student in voluntary four hours courses which included a short theoretical introduction, then in groups of two participants physician- patient discussions with role plays according to the stages of change by Prochaska and DiClemente, in each stage playing once the patient and once the physician. Student assessment was performed before and 3-4 weeks after the course by questionnaires and a five minute standardized patient situation for blinded offline analysis.
Results: A huge increase in knowledge could be measured: before the course a mean (SD) of 10.6 (2.7) out of 29 could be answered, and after the course 19.2 (3.6) ($p < 0.0005$). With questions aimed at attitude assessment a much higher motivation to assess patients on smoking behaviour and to council patients was attained (+36% on a visual analog scale, $p < 0.0005$). Blinded offline video analysis revealed a more flexible, more interactive, more empathic, and more relaxed type of standardized patient interview 3–4 weeks after the course leaving the patient more his own responsibility concerning his smoking behaviour compared to the interview before the course (blinded visual analog scale rating of two reviewers; p always ≤0.05), and the general judgement of the interview was also improved by 19% ($p < 0.0005$) on a visual analog scale compared to before the course. Spontaneous feedback underlined the need and motivation of the students for such a course.
Conclusion: A voluntary 4 hours pragmatic smoking intervention course for medical students at the Saarland University was effective to increase knowledge, skills and attitude towards smoking counselling in our short-term evaluation.

P144

A potential role for DEFA4 in the induction of steroid-induced adrenal insufficiency in patients with chronic obstructive pulmonary disease

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Background: A 14-day course of systemic corticosteroids is standard of care in the management of acute exacerbations of chronic obstructive pulmonary disease (AECOPD). However, supraphysiological intake of steroids has serious adverse effects including the suppression of the hypothalamic-pituitary-adrenal (HPA) axis. In clinical practice, a corticotropin (ACTH) test is used as surrogate to detect adrenal insufficiency. The aim of this study was to

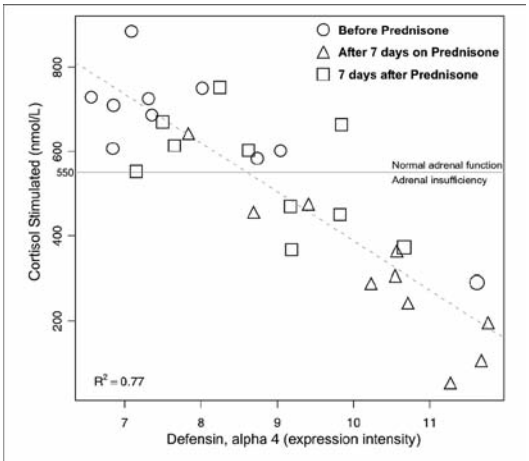

Figure 1: Correlation between the concentration of ACTH-stimulated cortisol and the DEFA4 gene expression in function of prednisone treatment. A normal response to ACTH test was defined as a stimulated plasma cortisol concentration > 550 nmol/L.

investigate gene expression variation in blood induced by a 14-day course of systemic corticosteroids treatment in patients with AECOPD, and to determine the mechanisms implicated in HPA axis suppression.
Methods: Peripheral blood gene expression profiling of 10 steroid-naïve patients with AECOPD was performed by microarray analysis (U133A, Affymetrix) before and after ACTH stimulation and in a time course (before prednisone, after 7 days on prednisone, and 7 days after prednisone). Genes associated with adrenal insufficiency were identified using between group analysis (BGA).
Results: At the end of prednisone treatment, only 1 of 10 patients had a normal / adequate rise of cortisol after ACTH stimulation. Seven days after prednisone treatment, 4 of 10 individuals still had adrenal insufficiency. The defensin alpha 4 (DEFA4) gene expression correlated with stimulated / peak cortisol levels, and, thus, HPA function (Figure 1; R-square = 0.77, p <0.01). The peptide DEFA4 is thought to antagonize steroid signaling by competing with ACTH for its receptor binding on cortisol-producing adrenal gland cells.
Conclusion: DEFA4 gene expression predicted the level of ACTH-stimulated cortisol, and might be used as a marker of HPA axis function. In addition, DEFA4, a putative antagonist of steroid signaling, might play a pivotal role in the maintenance of adrenal function during steroid treatment, but confirmation is warranted.

P145

Use of B-type natriuretic peptide in the risk stratification of acute exacerbations of chronic obstructive pulmonary disease

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Background: In patients with chronic obstructive pulmonary disease (COPD) prognosis might be determined at least in part by the extent of cardiac stress induced by hypoxia and pulmonary arterial hypertension.
Methods: B-type natriuretic peptide (BNP), a quantitative marker of cardiac stress, was determined in 208 consecutive patients presenting to the emergency department with an acute exacerbation of COPD (AECOPD). The accuracy of BNP to predict death at 2-years follow up was evaluated as the primary endpoint. The need for intensive care and in-hospital mortality were determined as secondary endpoints.
Results: BNP levels were significantly elevated during the acute exacerbation compared to recovery (65 pg/ml [34–189] vs. 45 pg/ml [25–85], p <0.001), particularly in those patients requiring ICU treatment (105 pg/ml [66–553] vs. 60pg/ml [31–169], p = 0.007]. In multivariate Cox regression analysis BNP accurately predicted the need for ICU care (HR 1.13; 95%CI 1.03–1.24 for an increase in BNP of 100 pg/ml; p = 0.008). In a receiver operating characteristic (ROC) analysis to evaluate the potential of BNP levels to predict short- and long-term mortality the area under the curves was 0.55 (SD 0.71, 95% CI 0.41–0.68) and 0.56 (SD 0.53; 95%CI 0.45–0.66) respectively.
Conclusions: In patients with AECOPD, BNP levels independently predict the need for intensive care. However, BNP levels failed to adequately predict short-and longterm mortality in AECOPD patients.

P146

Single photon emission tomography combined with computer tomography (SPECT/CT) for assessment of COPD patients for lung volume reduction surgery

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Assessment of COPD patients for lung volume reduction surgery includes body plethysmography, 6 minute walk, echocardiography, lung perfusion scintigraphy and computer tomography. Decrease of hyperinflation with improved diaphragmatic function is the main aim of lung volume reduction surgery. Patients with marked heterogeneity of lung emphysema show a better outcome than those with homogeneous emphysema. Therefore morphology of emphysematous changes on CT scan and perfusion patterns on scintigraphy are used to choose the best parts for lung resection. Recently a hybrid system (SYMBIA T2; Siemens) has been introduced allowing a combination of CT morphology with nuclear imaging. Posterior, anterior, right and left lateral and four oblique views are obtained with a dual head scanner with 128 projections over 360 C per head, a 128x128 matrix and 25 s/imaging stop, utilizing a low energy high resolution collimator. Planar scans consisted of posterior, anterior, right and left lateral and four oblique images. 183 MBq99mTc-MAA is injected and low dose CT scan with 8mm sections performed followed by 3 D picture fusion. Twenty COPD patients regarded as candidates for lung volume resection surgery were analysed by SPECT/CT. There were 11 (55%) male and 9 (45%) female patients with a mean age of 62.4 years ranging from 40 to 73 years. Mean FEV_1 was 0.82 litres (0.48 to 1.12) and 29.8% predicted (18% to 44%), total lung capacity 7.60 litres (5.94 to 10.44) and 133% predicted (109 to 148%), residual volume 4.83 litres (3.21 to 6.60) and 227% predicted (141 to 366%) and respectively. Mean diffusion capacity was 34.7% predicted (20 to 56%) and mean six minute walk distance was 329 metres (120 to 420). There was a drop in oxygen saturation of more than 10% in 15 out of 20 patients. SPECT/CT allowed to well define the emphysematous hypo-perfused lung regions and to localize the best parts for potential resection. There was marked heterogeneity in 15 cases (75%) predominantly in the upper lobes in 9 cases, lower lobes in 4 cases and marked left to right heterogeneity in 2 cases. SPECT/CT at three months follow-up of the first patients who underwent lung volume resection surgery after being assessed with this protocol will be presented.
Conclusion: SPECT/CT allows to better select regions of lungs for resection in COPD patients who are candidates for lung volume reduction surgery. If this method is capable to improve functional outcome is not yet known.

P147

A randomised, controlled trial of bosentan in severe COPD

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Background: Pulmonary hypertension at rest and during exercise is a frequent complication in the natural history of COPD. Endothelin-1 plasma levels are increased in COPD. We hypothesized that the use of the dual endothelin receptor antagonist bosentan can decrease pulmonary vascular resistance, enhance oxygen delivery, and thereby improve exercise capacity in patients with severe COPD.
Methods: In this double-blind, placebo-controlled study, 30 patients with severe or very severe COPD were randomly assigned in a 2:1 ratio to receive either bosentan or placebo for 12 weeks. The primary endpoint was change in the 6-minute walking distance. Secondary endpoints included changes in Borg dyspnoea index, WHO functional class, health-related quality of life, lung-function parameters, systolic pulmonary arterial pressures, maximal oxygen uptake during exercise testing, and regional perfusion pattern on single photon emission tomography (SPECT).
Results: As compared to placebo, patients treated with bosentan during 12 weeks showed no significant improvement in exercise capacity as measured by the 6-minute walking distance (331 m [123] versus 329 [94], respectively, p = 0.474). There was no change in Borg dyspnoea index, WHO functional class, lung function, systolic pulmonary arterial pressure, maximal oxygen uptake, and regional perfusion pattern on SPECT (all p = ns). Conversely, arterial oxygen partial pressure decreased (p = 0.029) and health-related quality of life deteriorated significantly in patients assigned bosentan (p = 0.039).
Conclusions: The oral administration of a dual endothelin receptor antagonist not only failed to improve exercise capacity but also deteriorated hypoxemia and functional status. Therefore, therapy with bosentan cannot be recommended in patients with severe COPD.

P148

Bronchodilator response in residual volume in irreversible airway obstruction

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Background: Although, reversibility of airway obstruction, as defined by improvement of the forced expiratory volume in one second (FEV_1) and/or the forced vital capacity (FVC), is lacking in patients with COPD, they clearly seem to benefit from treatment with inhaled bronchodilators.
Aims: To assess the response pattern of the residual volume (RV) compared to FEV_1 after bronchodilation in patients with reversible and irreversible airway obstruction.
Methods: Changes in static lung volumes were compared with improvement in dynamic lung volumes in 396 consecutive patients undergoing reversibility testing with repeat bodyplethysmography. Reversibility was defined as improvement of FEV_1 >200 ml and >12% after inhalation of fenoterol hydrobromide.
Results: Irreversibility was found in 297 out of 396 patients with airway obstruction.

Except for total lung capacity (TLC), all parameters (residual volume (RV), vital capacity (VC), forced inspiratory vital capacity (IVC), forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and the FEV_1/VC ratio showed statistically significant changes after bronchodilatation in 396 patients.
By performing a multiple linear regression model adjusted for age, sex and BMI, there were a non-linear relationship between change in FEV_1 or change in VC compared to change in RV after bronchodilation. There is a highly significant contribution of the spline to the regression model ($p < 0.001$) indicating a nonlinear relationship between change in FEV_1 and change in RV. Below a change in FEV_1 of about 0.1 L, change in RV remains constant whereas a change in FEV_1 value above 0.1L, chanage in RV decreases. There is also a nonlinear dependency of change in RV to change in VC ($p < 0.001$).
Conclusion: In summary, in patients with irreversible airway obstruction changes in RV cannot be predicted by changes in FEV_1 or VC after bronchodilation. Therefore, spirometric assessment should be completed by bodyplethysmography.

P149

Efficacy and tolerability of EPs® 7630 tablets in adult patients with acute bronchitis – a randomised, placebo-controlled, double-blind, dose-finding study

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Background: EPs® 7630, a herbal drug preparation from the roots of Pelargonium sidoides (1:8-10), extraction solvent: ethanol 11% (w/w), has been successfully used as EPs® 7630 solution (Umckaloabo®; ISO Arzneimittel, Ettlingen, Germany) for several years by adults and children at an age of 1 year or above with various infections of the ENT and respiratory tract. This study investigated the efficacy and tolerability of EPs® 7630 tablets in patients >18 years with acute bronchitis.
Patients and methods: 405 patients (mean age: 40 years, female: 70%) were randomly assigned to one of 4 treatment groups (3x1 tablet/day of 10 mg, 20 mg, 30 mg EPs® 7630, dried, or placebo) for a total treatment-period of 7 days. The primary efficacy variable was the change in the total score of bronchitis-specific symptoms (BSS) from Day 0 to Day 7. The BSS score consists of 5 symptoms: coughing, sputum, pulmonary rales at auscultation, chest pain while coughing, and dyspnoea, which are rated on a 5-point scale from 0 (not present) to 4 (very severe).
Results: Between Day 0 and Day 7, the BSS total score decreased by 2.7 (placebo group), 4.3 (30 mg), 6.1 (60 mg) and 6.3 points (90 mg), respectively ($p < 0.0001$ for the test for dose-response relationship, and for all pair-wise comparisons between the active treatment groups and placebo). The superiority of EPs® 7630 tablets versus placebo was also confirmed in the responder analyses and in all other secondary endpoints. EPs® 7630 tablets were very well tolerated; serious adverse events were not reported.
Conclusion: This study demonstrated both statistically significant and clinically relevant superiority of all three tested dosages of EPs® 7630 tablets versus placebo. All dosages of EPs® 7630 tablets were very well tolerated.

P150

Change of exercise challenge test and bronchial hyperresponsiveness to dry powder mannitol in adolescents during optimised asthma treatment

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Clinical background: Exercise-induced symptoms are frequently found in asthmatic children. Therefore, treatment response to asthma therapy could be monitored by repeated exercise challenge tests. Using osmotic aerosols such as dry powder mannitol might be an alternative to assess treatment response.
Study aim: Study aim was to investigate the change of exercise-induced bronchial hyperresponsiveness (EIB) and bronchial hyperresponsiveness (BHR) to dry powder mannitol during a two week period of optimized asthma treatment in children with exercise-induced asthma.
Methods and Patient characteristics: 26 patients (21 male, 5 female) aged 8 to 20 years with physician diagnosed asthma were recruited from the Alpine Children's Hospital Davos (Switzerland). All patients underwent a lung function, an exercise challenge test (ECT) using a treadmill and a mannitol challenge test on two different days (tests day 0). Response to ECT was positive when a drop in FEV_1 of >15% after challenge was reached. Response to mannitol was positive when a provoking dose caused a 15% fall in FEV_1 (PD15). The response-dose ratio (RDR = % of maximal fall in FEV_1/maximal dose mannitol given) was then calculated.
Optimized treatment included inhaled corticosteroids (at least 250 mg fluticasone or 400 mg budesonide per day) and inhaled bronchodilators for the first 7 days when ECT and MCT were repeated (tests day 7). For the next 7 days montelucast was added and ECT and MCT were repeated again (tests day 14).
Results: Lung function at baseline was normal in all patients with a mean FEV_1 of 111% predicted (± 16.3%) and a mean FVC % of 115.3% predicted (±17.4%).
At baseline (day 0), 14 patients had a positive ECT, 8 of them had also a positive mannitol challenge test.
With optimized asthma therapy, maximum fall in FEV_1 after ECT decreased significantly between day 0 and day 14 (difference of median 16.8%; 95% CI 11.9 to 31.1; $p < 0.001$). Between day 0–7 and 7–14 a trend towards a decrease could be shown but no significant difference. There was also a trend of RDR mannitol to decrease with optimized asthma therapy over the period of 14 days, however the difference was not statistically significant (difference of median –0.018785; 95%CI –0.056615 to 0.006350; $p = 0.1089$).
Conclusion: During optimized asthma therapy, there is a significant improvement of exercise challenge test and a trend towards improvement in mannitol challenge test in adolescents with exercise induced asthma.

P151

Technical aspects of exhaled NO (FeNO) measurement using a hand-held device (NIOX MINO) in daily practice and effect of pulmonary function testing on FeNO

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Background: FeNO is a marker of eosinophilic airway-inflammation and can be used to guide asthma treatment. With the NIOX MINO, a hand-held device, FeNO-measurement can easily be performed. Nevertheless, there are few data of technical practicability in daily practice.
Aim: 1) To determine the feasibility and repeatability of FeNO measurement in asthmatics. 2) To evaluation FeNO in asthmatics before and after pulmonary function testing (PFT).
Methods: 1) From 2/07 to 4/07 (period A) 28 asthmatics performed two FeNO measurements with NIOX MINO with only a few minutes difference in-between measurements. 2) From 6/07 to 10/07 (period B) 32 asthmatics performed one measurement before and the second after PFT. Values were compared with Bland-Altman analysis.
Results: Mean age was 49 years (95% CI 43–54 y, range 17–76 y) in group A and 46y (40–52 y, 17–76 y) in group B. Mean FEV_1 was 2.0L (1.7–2.4L), 75% (65–84%) predicted in group A and 2.4 L (2.1–2.8 L), 77% (69–84%) in group B. 14 (50%) patients had a FEV_1/VC below 0.7 in group A with significant reversibility in 11 (79%) and 21 (66%) in group B with significant reversibility in 10 (48%). In booth groups there was a significant correlation ($p < 0.0001$) of the two FeNO measurements with a correlation-coefficient of 0.97 (0.95–0.99) and 0.96 (0.92–0.98), respectively. In group A in 17 (61%) first FeNO was lower (mean difference 4ppb, 1-7ppb) and in 5 (18%) higher (mean difference 5ppb, 1–9 ppb). In group B in 15 (47%) FeNO was lower (mean difference 7.6ppb, 3.4–11.8 ppb) before PFT and in 13 (41%) patients higher (mean difference 3.8ppb, 2.3–5.3 ppb). In booth group, neither grade of obstruction nor reversibility influenced this difference. The bias of FeNO in group A was +1.5 ppb (–1–3.9) with a lower and upper 95% limit of agreement of –10.8 ppb and +13.7 ppb. The bias in group B was +2 ppb with a lower and upper 95% limit of agreement of –12.9 ppb and +16.9 ppb. Most patients were capable to perform FeNO-measurements.
Conclusion: FeNO measurement with the hand-held device NIOX MINO can easily be performed in the pulmonary function lab. Correlation of FeNO in booth group was high. There was a low bias in booth group, nevertheless, 95% limits of agreement was up to 14ppb. These results are comparable to the few published data concerning accuracy and repeatability of this hand-held nitric oxide analyzer. PFT-measurement influences FeNO, nevertheless in booth direction (in some higher, in some lower after PFT).

P152

A successful treatment of mediastinal fibrosis with low-dose prednisone combined with mycophenolate mofetil

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Background: Mediastinal fibrosis is associated with several syndromes such as retroperitoneal fibrosis, Reidel's thyroiditis, sclerosing cholangitis, pulmonary hyalinizing granuloma or multifocal fibrosclerosis. These syndromes are believed to be different manifestations of the same disease. It's a rare disease. It is estimated that its incidence is 1/1'000'000 person-year and its prevalence is 1.38 cases/100'000 inhabitants. It is idiopathic or secondary seen in autoimmune diseases, infections or drug induced. The age at disease onset peaks between 50 and 60 years. Pathohistological research show a mixture of chronic inflammation with B- and T-cells and fibrosis. Immunosuppressive therapy with prednisone was the basic therapy regimen for years next to surgery in cases of complications. Because of the rarity of this disease there are lacking endemic studies and no evidence based therapy is determined. Several case report studies show successful treatments with newer immunosuppressive agents.
History: We present a case of a 67 year old man with mediastinal fibrosis. On his holidays in spain he was suffering from a syncope. After returning home, a progressive dyspnoe developed. Further examinations revealed a aortic stenosis and a stenosis of the right coronary artery. A surgical intervention was planed. The preoperative evaluation with a CT-scan showed a soft-tissue density mediastinal mass surrounding the aorta. The histopathological examinations were consistent with periaortitis as seen in retroperitoneal fibrosis and mediastinal fibrosis. Laboratory findings showed normal function of the kidneys and autoimmune antibodies were normal.
Therapy and follow up: Because of his diabetic state, he was treated with a steroidsparing agent. We tried mycophenolate mofetil because of its relatively few adverse reactions. He was treated with prednisone 10mg/d and mycophenolate mofetil 2 g/d.
A follow up 3 months after starting the therapy showed a regression of the mediastinal and retrocrural mass. No signs of aneurism were detectable. The medication was well tolerated, the diabetes was under control.
Conclusion: Mycophenolate mofetil and low dose steroids is a safe and efficacious therapy in mediastinal fibrosis.

P153

Engineered nanoparticles for gene delivery in a bleomycin-injured lung fibrosis model

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Background: Pulmonary fibrosis is a progressive disease with a poor prognosis. To date the mechanisms of this devastating disease are still unclear and no specific therapy exists. Increasing evidence suggests that the changes present in pulmonary fibrosis result from sequential alveolar epithelial injury and abnormal alveolar wound repair. Complex cell interactions and mediators including growth factors are involved in this repair process. Hepatocyte growth factor (HGF) has been implicated in the prevention and also treatment of pulmonary fibrosis. Our own results have shown that pulmonary fibrosis was attenuated by in vivo electroporation-mediated gene transfer of hHGF (Gazdhar A et al, Am J Physiol Lung Cell Mol Physiol. 2007;292(2):L529–36). For future clinical applications, gene therapy applications require safe and efficient methods for gene delivery. Anticipated applications for engineered nanoparticles (NP) in medicine include the use of bioengineered NP for novel therapeutic or diagnostic approaches. Biocompatible NP may therefore represent an efficient, safe, yet minimally invasive method for gene delivery in the respiratory tract. We herein present the development of a protocol for NP-based gene transfer to the bleomycin injured lung.
Methods: Super-paramagnetic iron oxide NP (SPION) were produced that contain an iron oxide core coated with a fluorochrome-labelled polymer shell (PVA). SPION (250 µg) were administered in a volume of 250 µl unilaterally to the left lung through intra-tracheal intubation 7 days after bleomycin-induced lung injury in a rat model. The experiments were terminated at 24 hrs post NP instillation and NP deposition in fibrotic lung tissue was evaluated by confocal microscopy.
Results: In preliminary proof-of-concept studies, confocal microscopy showed homogenous NP deposition in the lung confined to the alveolar epithelium and macrophages without inducing marked alveolar inflammation. Further characterisation by EM is underway for detailed characterisation of SPION-cell interactions.
Conclusions: Initial proof-of-principle experiments showed an preferential deposition of NP to alveolar epithelial cells that are a target for novel gene therapies in lung fibrosis. Engineered NP may therefore provide a novel approach for gene delivery in pulmonary fibrosis.

P154

Comparison of batch adjustment methods for the analysis of gene expression microarray data

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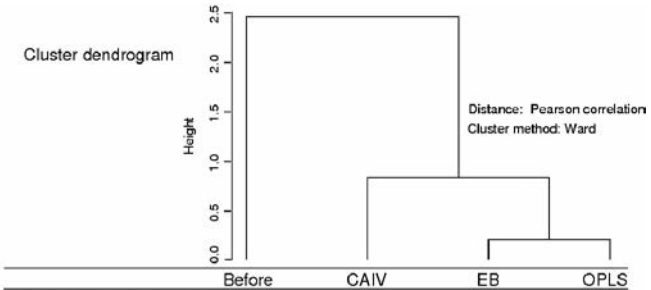
Background: Microarray experiments are often performed in batches due to the characteristics of sample acquisition (multi-institutions, cell culture experiments, different microarray platforms), or due to practical reasons (load capacity for RNA extraction, labeling or hybridization). As a consequence microarray datasets are often affected by non-biological experimental variance or "batch effects". Different batch adjustment methods have been proposed aiming to reduce this technical variance.
Methods: We benchmarked 3 different batch adjustment methods, which are Correspondence Analyses with respect to Instrumental Variables (CAIV), Empirical Bayes (EB) and Orthogonal Projections to Latent Structures (OPLS). Calculations were done on 4 publicly available lung cancer datasets, which are Bhattacharjee et al. (2001), Beer et al. (2002), Bild et al. (2006) and Yap et al. (2005). Data pre-processing by these algorithms were compared using multivariate correlation coefficients (RV). The gain obtained in terms of identification of differentially expressed genes and classification accuracy was assessed.
Results: CAIV, EB and OPLS successfully adjusted batch effects. Data pre-processed using CAIV and EB showed a higher accuracy to identify differentially expressed genes. OPLS showed a higher classification accuracy than CAIV and EB. The choice of the appropriate algorithm also depends on the experimental design. For CAIV and EB, specific batch information is required. In contrast to EB, CAIV can simultaneously handle nested batch information. OPLS, which requires a priori phenotypic information, may be more prone to overfitting.
Conclusion: Batch effects can significantly correct, but also distort the results of microarray data analysis. Different batch adjustment methods have different characteristics, which impact on the results of downstream statistical analysis. Among the three methods, CAIV appears particularly interesting due to its high flexibility.
Figure 1: Hierarchical clustering comparing the RV correlation coefficient which measures the similarity between two datasets before and after batch adjustment pre-processing. CAIV, EB and OPLS perform equally well and give comparable result. The integrated table summarises the characteristics of the 3 methods in question ("+" efficient, "0" indifferent, "–" inefficient or "NA" not applicable).



	Before	CAIV	EB	OPLS
Identification of dysregulated genes	–	+	+	–
Classification accuracy	–	0	–	+
Phenotypic information required	NA	+	+	–
Batch information required	NA	+	+	+
Handles several batches	NA	+	NA	0
Handles nested batches	NA	+	NA	0
Time consuming	NA	0	+	–

P155

Investigation of immune responses to engineered nanoparticles

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Background: Recent years have shown an accelerating pace in nanotechnology research and applications, expected to provide significant advances in the diagnosis and treatment of many diseases. Anticipated applications include drug/gene delivery, diagnostics, imaging, and production of biocompatible materials. Though epidemiological and experimental data on combustion-derived nanoparticles (NP), such as diesel exhaust particles, showed that exposure is associated with a wide variety of adverse health effects, there is an vast and constantly increasing body of research performed for the controlled use of biomedical NP. Though numerous studies have addressed toxicological aspects of NP, very few general trends have been established that enable a prediction of the immune response a given NP will generate. Different parameters such as antigenicity, adjuvant properties, inflammatory potential, and internalisation mechanisms determine the immune-stimulatory effect of a given NP. Dendritic cells (DC) are key antigen-presenting cells involved with immunity and induction of T cell responses. The current study reports preliminary results of in vitro studies investigating the effect of NP exposure on DC activation and function.
Methods: Monocyte-derived DC (MDDC) were exposed for 24h to super-paramagnetic iron oxide NP (SPION) coated with a fluorochrome-labelled polymer (PVA). Surface phenotype of DC and NP uptake were monitored by FACS. Additional investigations will include confocal and electron microscopy and functional assessment of T cell stimulatory activity by autologous CD4+ T cell proliferation and cytokine profiles.
Results: In initial proof-of-concept studies, exposure of MDDC to engineered NP resulted in the following changes: (1) minimal cell death; (2) NP uptake induced a dose-dependent increase in detectable fluorescence; and (3) minimal changes in surface phenotype (CD80, CD83, CD86, myeloid DC, or plasmacytoid DC markers).
Conclusions: Engineered NP induced minimal surface phenotypic changes of MDDC. Future studies will investigate NP-cell interactions and functional effects to further characterise immune effects of biomedical NP. Specifically designed NP may constitute the basis for novel therapeutic and diagnostic applications in the respiratory tract.

P156

Acute effects of short-term exposure to organic particulate matter on endothelial function

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Introduction: Ambient air pollution by particulate matter (PM) is associated with increased cardiovascular morbidity and mortality. The PM size determines its biological action: PM10 (aerodynamic diameter <10 μm) may reach the alveolar space, whereas PM1 (aerodynamic diameter <1 μm) may enter the bloodstream. We investigated the composition of hay dust and the acute effects after its inhalation on peripheral microvascular endothelial function and circulating markers of endothelial function.
Methods: Healthy young volunteers were exposed to PM inhalation during hay storage for 2 hours. PM10 was collected by an aerosol sampler and particles were counted by a spectrometric dust monitor. Colony forming units (cfu) of fungi were counted on dust filters. In all subjects peripheral microvascular endothelial function using RH-PAT (Reactive Hyperaemia Peripheral Arterial Tonometry), and markers of endothelial function were assessed in the morning before and after PM exposure.
Results: Concentrations of PM10 ranged from 2489 μg/m³ to 8726 μg/m³. The majority of it was PM1 (79–93%) with an average diameter around 0.25 μm. Fungi were very abundant with a maximum of 4.4 x 10^6 cfu/filter, corresponding to 89'600 cfu/m³ of filtered air. Von Willebrand factor was increased after PM exposure (94.31 vs 106.46, p = 0.026). E-Selectin was not affected. Peripheral microvascular endothelial function was similar before and after PM exposure.
Conclusions: Hay dust contains large amounts of fine and ultrafine PM. Early after PM inhalation increased levels of von Willebrand factor were observed, whereas microvascular endothelium-dependent vasomotion was not affected. Our results suggest a differential response of the endothelium to short-term PM exposure.

P157

In vitro comparison of curcuma longa extracts for their potential future use in chronic inflammatory lung diseases

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Background: Turmeric (curcuma longa) rhizome is used in traditional medicine to treat different inflammatory diseases. The polyphenolic curcumin is the most known active agent of this plant. However, its molecular mode of action is unknown. Other substances contained in turmeric could have significant biological activity. The aim of this study was to identify the most promising curcuma longa extract in terms of anti-inflammatory and anti-proliferative activity with least toxicity. Early Growth Response 1 (EGR-1) was studied as potentially mediating the anti-inflammatory activity of curcumin.
Methods: In collaboration with Vitaplant Inc. curcuma longa extracts were obtained by different percolation and maceration protocols. These extracts as well as commercially available curcumin were tested on promyelocytic leukemia cells for their anti-inflammatory activity. Cytotoxicity, anti-proliferative activity, and effect on extracellular matrix (ECM) deposition of curcuma longa extracts were measured by using respective biological cellular assays on primary lung fibroblasts from patients of the University Hospital Basel. The EGR-1 protein concentration was analyzed in function of curcuma longa extracts treatment.
Results: Compared to commercially available curcumin, which had significant toxicity, the most promising extract showed a lower cytotoxicity, a higher decrease of ECM deposition, and a similar anti-proliferative effect. Moreover, this promising extract presented a high anti-inflammatory activity which correlated with the EGR-1 levels measured (Fig. 1).
Conclusion: Significantly differing biological effects were observed among the different curcuma longa extracts. We hypothesize the implication of EGR-1 in the mediation of the anti-inflammatory effects of curcumin. The most promising extract exhibited properties worthwhile to be studied further for its potential use in patients with chronic inflammatory or fibroproliferative lung diseases.

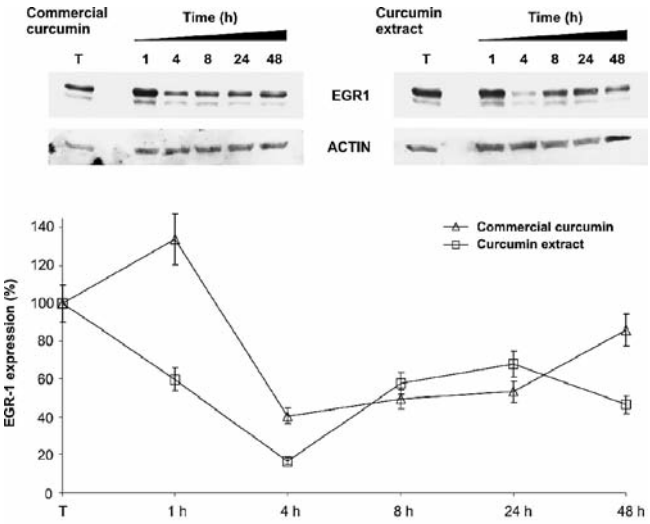

Figure 1: The treatment with curcumin led to a significant and early down-regulation of EGR-1 protein (Western blot analysis), which paralleled its anti-inflammatory activity (not shown).

P158

Aerosolised salbutamol accelerates the resolution of pulmonary oedema after lung resection

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Background: Ischemia-reperfusion injuries, fluid overload and cardiac insufficiency may all contribute to alveolar and interstitial lung edema. We hypothesized that aerosolized salbutamol would reduce extravascular lung water and improve oxygenation after lung resection, by stimulating epithelial fluid clearance and cardiovascular function.
Methods: This is a blinded, randomized, cross-over trial. We selected twenty four patients with risk factors for lung edema (>60 yrs, history of chronic alcohol consumption, prior radiation or chemotherapy, cardiac insufficiency, coronary artery disease, recent pneumonia and/or reduced diffusion capacity for carbon monoxide). Aerosolized drugs (salbutamol 5 mg vs. ipratropium 0.5 mg) were given on 2 consecutive trials, with a 6-hours washout period, on the day of surgery (POD 0) as well as on the first postoperative day (POD1). Before and 50 min after the end of drug administration, we determined the oxygenation index (PaO_2/FIO_2 ratio), the extravascular lung water index (EVLWI), the pulmonary vascular permeability index (PVPI) and the cardiac index (CI) using the single indicator thermal dilution technique.
Results: Complete data were obtained in 21 patients. On POD0, the EVLWI was increased compared with preoperative values (13.0 ± 3.8 vs. 9.1 ± 4.4, P <0.001); salbutamol treatment induced significant increases in PaO_2/FIO_2 ratio (+25 ± 13%) that was associated with decreases in EVLWI (–18 ± 10%, P <0.05) and in PVPI (–19 ± 10%, P <0.05) along with increased cardiac index (+23 ± 11%, P <0.05). On POD1, repeated nebulization of salbutamol induced significant increases in PaO_2/FIO_2 ratio and CI (+22 ± 10% and 19 ± 11%, respectively) whereas both EVLWI and PVPI remained unchanged. Nebulization of ipratropium bromide did not produce significant hemodynamic and respiratory changes on POD0 and POD1.
Conclusions: Aerosolized salbutamol accelerates the resolution of lung edema, improves blood oxygenation and stimulated cardiovascular function after lung resection in high-risk patients.

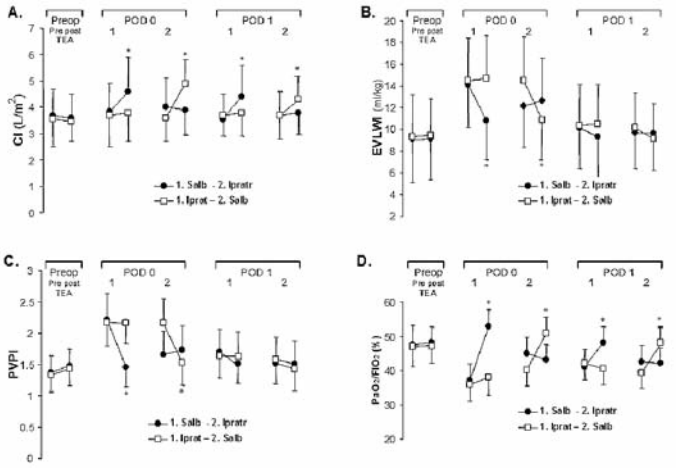

Table Hemodynamic and respiratory effects induced by thoracic epidural anesthesia and aerosolized Salbutamol vs. Ipratropium in patients undergoing lung resection

	Preop		POD 0				POD 1			
	Before TEA	After TEA	Before Salbutamol	After Salbutamol	Before Ipratropium	After Ipratropium	Before Salbutamol	After Salbutamol	Before Ipratropium	After Ipratropium
Hemodynamic variables										
MAP mmHg	89 (15)	76 (12)*	77 (15)	79 (16)	79 (16)	81 (16)	74 (18)	76 (14)	77 (20)	76 (25)
CI ml/m²/min	3.7 (1.1)	3.5 (0.8)	3.8 (1.0)	4.7* (1.2)	3.7 (0.9)	3.7 (1.0)	3.6 (1.0)	4.3* (1.2)	3.5 (0.6)	3.5 (0.9)
HR beat/min	75 (15)	67 (10)*	72 (14)	79 (16)*	74 (10)	75 (10)	76 (12)	79 (12)	76 (6)	77 (9)
SV ml	89 (27)	93 (27)	94 (33)	106 (34)*	89 (18)	87 (18)	88 (18)	97 (23)*	83 (14)	82 (18)
SVRI dyne x sec/m²/m²	1166 (330)	962 (238)*	862 (274)	720 (232)*	962 (330)	963 (311)	821 (266)	766 (182)	925 (170)	967 (191)
dP/dtmax mmHg/s	827 (402)	676 (271)	809 (358)	1024 (365)*	944 (436)	927 (366)	955 (198)	1122 (203)*	1004 (431)	1079 (402)
Respiratory variables										
ITDVI ml/kg	33.5 (10.5)	33.0 (9.5)	32.5 (8.9)	32.4 (8.9)	31.7 (8.6)	32.1 (8.3)	26.0 (7.8)†	26.8 (5.6)	26.7 (6.4)†	27.8 (5.3)
EVLWI ml/kg	9.0 (3.2)	9.1 (4.4)	13.2 (3.6)†	10.9 (3.6)*	12.5 (4.3)†	12.3 (4.6)	10.5 (3.5)	9.2 (3.3)*	9.8 (4.4)	9.9 (4.6)
PVPI	1.3 (0.3)	1.5 (0.3)	2.0 (0.4)†	1.5 (0.4)*	1.9 (0.4)†	1.9 (0.3)	1.6 (0.4)	1.4 (0.5)	1.6 (0.5)	1.5 (0.4)
PaO_2 / FIO_2 kPa/%	48.2 (8)	49.3 (4)	37.2 (5)†	51.7 (5)*	37.8 (4)†	39.7 (4)	39.2 (4)†	48.6(4)*	40.4 (5)†	41.7 (4)†

† P<0.05, compared with preoperative baseline (after TEA); * P< 0.05, compared with pre-intervention (TEA, Salbutamol or Ipratropium); TEA, Thoracic Epidural Anesthesia
MAP, Mean arterial Pressure; HR, Heart Rate; SV, Stroke Volume; SVR, Systemic Vascular resistance
PVPI, pulmonary vascular permeability index; GEDM, global end-diastolic volume index; EVLWI, extravascular lung water index; PaO_2 arterial oxygen partial pressure
FIO_2, fractional inspiratory oxygen.

P159

The chemokine receptor CCR7 on T-lymphocytes and its regulation by CCL19 and CCL21

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The chemokine receptor CCR7 is expressed on naive T lymphocytes and is the central regulator for trafficking of these cells into and within secondary lymphoid organs (SLO). Its two agonistic ligands CCL19 and CCL21 are produced by T zone reticular cells (TRCs) in SLOs. Human T cells react quite differently to the two CCR7 ligands CCL19 and CCL21. Stimulation by CCL19 but not CCL21 leads to pronounced receptor desensitization through receptor phosphorylation and beta-arrestin binding. Furthermore, CCL19, but not CCL21, evokes receptor internalization and is then degraded, while CCR7 is recycled to the cell surface. The differential behavior of CCL19 and CCL21 is surprising given that the two CCR7 ligands have comparable binding affinities and induce G protein activation, calcium mobilization and chemotaxis with similar efficiency.
Up to now the regulation of CCR7 on murine T lymphocytes has been poorly characterized. Recently, a new antibody against murine CCR7 that recognizes free as well as occupied receptor has become available. Using this and a CCL19-Fc fusion protein, we can distinguish free and occupied CCR7 by flow cytometry. We will present the first in vivo study addressing the question of how CCR7 adjusts to the different levels of chemokines the T cells encounter on their journey through blood and lymphoid organs in wildtype, CCL19- and CCL19/21-deficient mice. We will also show how cell migration is influenced by the differential surface availability of the receptor CCR7. Finally, evidence will be provided for the rapid adaptation of T cells to the environment, both in vitro and in vivo.

P160

Trapping of in vitro activated human T-lymphocytes by mesenchymal multipotent stromal cells (MSC) in a closed environment is required for their inhibition

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Background: MSC are post natal stem cells that are able to differentiate into many cell lineages and can control allogenic T lymphocyte (TL) proliferation. Both soluble and cell-bound mediators are involved in the process. Recently we showed that MSC-driven inhibition was associated, in vitro, with TL transmigration through the MSC layer (J Cell Physiol ahead pub, sept 2007). We further investigated this issue to assess whether other mechanisms were involved in TL inhibition.
Methods: TL purified by negative immunoselection on paramagnetic beads were labeled with CFSE and seeded over allogenic MSC obtained from femoral heads removed from patients undergoing total hip replacement surgery. Cultures were either not stimulated, or stimulated with TL mitogens (PHA+IL-2, anti-CD3 and -CD28 mAb immobilized on beads). MSC were plated in regular 24-well plates, or in chambers whose basement consisted in 0.4 or 3.0-micron pore-sized membranes (mm). TL proliferation was assessed after 5 days by monitoring CFSE dilution.
Results: In coculture with 3 mm, TL migrated from 48 hours on through the MSC layer and the membrane. After 5 days of culture with mitogens TL recovered in the lower chamber had significantly proliferated, and were only marginally inhibited by MSC. When 0.4mm were used, TL could not cross the membrane and remained tightly associated with MSC, as trypsination was necessary to harvest them. Despite their localization nearby the MSC, these TL responded efficiently to mitogens, and were only marginally inhibited by MSC. By contrast duplicate cultures performed in regular hard plastic-bottomed wells showed that MSC were fully inhibitory in such a setting.
Conclusion: These data demonstrate that TL inhibition occurs only when transmigration targets TL under MSC bound to a waterproof surface. When TL transmigrate under MSC that adhere to a membrane allowing metabolite exchange (0.4 mm), or when TL can run away from MSC after transmigration (3 mm), no significant inhibition ensues. This suggests that TL have to be sequestrated for at least 48 hours in a hermetically sealed volume under MSC in order to be inhibited. This is consistent with the observation that MSC induce TL inhibition via the activation of the intracellular enzyme indoleamine 2,3 oxygenase that metabolizes L-tryptophan. Small sealed volumes under – or engulfed in – MSC certainly represent a privileged environment to induce efficient L-tryptophan depletion and LT inhibition.

P161

Building a lymph node in vitro

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Adaptive immune responses are only efficiently initiated in the T-zone of secondary lymphoid organs where antigen-specific T-cells encounter antigen-bearing dendritic cells (DC). These encounters are facilitated by a 3-dimensional (3D) network of fibroblastic reticular cells (FRC) present throughout this zone. Incoming DCs adhere to FRCs and T cells are physically guided in their migration by this network. Importantly, FRCs directly regulate DC and T cell migration by producing the two CCR7 ligands. In addition, FRCs were recently shown to produce the cytokine IL-7, a critical T-cell survival factor. Therefore it has become apparent that FRCs play an important role in adaptive immunity. However, their biology and precise role in T cell priming are poorly understood.
We established immortalized FRC clones from lymph nodes of p19-/- and p53-/- mice and FACS analysis showed that these cells express a surface profile comparable to ex vivo FRCs. These cells proliferate rapidly in vitro and provide an easy source of FRCs to further study their biology. As it became apparent in recent years, that cells cultured in 3D models reflect much more the biology of cells in vivo, we established a 3D culture system with these FRC lines. When grown in a collagen-containing sponge the cells build their characteristic network and show a morphology comparable to their counterparts in lymph nodes. By co-culturing FRCs together with T cells and DCs it should be possible in future to reconstruct the unique microenvironment of the T zone. Such a system should be easy to manipulate and to study T-cell priming by DCs in a close to physiological context.

P162

Human cytomegalovirus efficiently infects porcine endothelial cells

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Background: Reactivation of human cytomegalovirus (hCMV) is a potential risk following the clinical application of pig-to-human xenotransplantation. We investigated the capabilities of various hCMV strains to infect porcine endothelial cells (pEC) and putative immunological consequences thereof.
Methods: pEC derived from iliac artery, vena cava, microvasculature, and aorta were incubated with the hCMV laboratory strains AD169, TB40/E and TB40/F or a clinical isolate. Viral replication kinetics and evolution of cytopathology were analyzed in detail. Consequences of pEC infection on human NK cell activation were evaluated by a xenostimulation assay assessing NK cell IFNg secretion and cytotoxicity.
Results: Infection was evident and a maximum percentage of infected cells was reached at a MOI of 10. hCMV replicated in all tested pEC types, with a fraction of infected cells ranging from 1% to 50%. AD169 infection of 2A2 (microvascular EC) resulted in cytopathic effect (CPE) development by 3 dpi and in lysis of 70% of the cells at 7 dpi. Contrary, no CPE was observed in PED (aortic EC) up to 15 dpi. Infection of 2A2 with TB40/E was non-lytic and resulted in accumulation of virus. It was further demonstrated that infected pEC supported a complete replication cycle and produced viral progeny. Preliminary results showed that coculture of human NK cells and infected pEC resulted in increased NK killing and IFNg production.
Conclusions: In summary, these findings provide evidence that pEC are permissive and support the complete productive replication cycle of hCMV. Moreover, pEC infection leads to modification of the xenogeneic cytotoxicity mediated by human NK cells. Our findings allow a better estimation of the potential role of hCMV cross-species infection following xenotransplantation and may be crucial to guide future clinical trials.

P163

Human cytomegalovirus infection of porcine endothelial cells induces increased adhesion receptor expression and human leukocyte adhesion

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Introduction: Human cytomegalovirus (HCMV) frequently reactivates in immunosuppressed transplant recipients and may trigger graft rejection. Following infection, the endothelium is altered and contributes to host leukocyte recruitment and activation of the immune response. Cross-species productive infection of porcine endothelial cells (pEC) by HCMV has been demonstrated.We investigated the effect of HCMV infection on adhesion molecule expression on pEC and on the interactions between human leukocytes and pEC.
Material and methods: Primary pEC were inoculated with the HCMV laboratory strains AD169 or TB40/E at different multiplicity of infection (MOI). UV-inactivated or heparin-treated virus were used as control inoculum. Infection was assessed by immunocytological staining for immediate early antigen (IEA) and quantitative PCR. Phenotype of pEC was analyzed by flow cytometry. Adhesion of human PBMC on pEC was investigated under physiological shear.
Results: At 1 day post inoculation (dpi) with a MOI of 1, cellular entry was evident by PCR, and distinct nuclear IEA staining was observed in 20% of pEC infected with AD169 and 10% with TB40/E. Preincubation of the virus with heparin lead to a strong blocking of infection. At 1 to 2 dpi, HCMV infected pEC showed significantly upregulated surface expression of VCAM-1 (CD106), and to a lesser extent of E-Selectin (CD62E). In agreement, adhesion of human monocytes, NK, NK/T, T, and B cells was increased on HCMV-inoculated pEC as compared to control.
Conclusion: HCMV productively infects pEC in vitro leading to increased adhesion receptor expression and human PBMC adhesion. Thus, in pig-to-human solid xenotransplantation HCMV infection may alter the porcine endothelium rendering the xenograft more susceptible to human leukocyte adhesion and infiltration, resulting in an enhanced immune response. In conclusion, recipient-derived HCMV infections need to be carefully considerated in the context of xenotransplantation.

P164

CD93 is required for long-term functions of plasma cells in the marrow niche

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CD93 is expressed during early B-cell development. Here we show that this surface molecule is re-expressed during the late differentiation stages of antibody secreting cells after both in vitro and in vivo B cell activation with T-independent and T-dependent stimuli. This occurred via 2 pathways, either before or after CD138 or Blimp-1 expression. Moreover, we found that decreased cell cycle activity, isotype-switched antibody secretion, and modification of the transcriptional network correlated with the more mature plasma cell stage of CD93/CD138 double positive cells. No expression was found on germinal center or memory B cells. Regulation of CD93 expression was analysed using Blimp-1-/-, Aiolos-/- and OBF-1-/- mice, which are known to present a defect a different stages of plasma cell maturation.
In order to determine whether CD93 plays a role in humoral response, CD93 knockout mice were analysed. No difference was observed between CD93 deficient mice and wild-type counterparts in the early phases of the immune response. However, at later time points a significant decrease in antibody levels was observed in CD93 deficient mice. In vivo transfer experiments showed that plasma cell migration was normal but that their persistence in the bone marrow was strongly reduced in the absence of CD93. Our results suggest that CD93 is non-redundant for the maintenance of plasma cells in the bone marrow niche.

P165

Monomeric and dimeric IgG fractions from intravenous immunoglobulin (IVIg) show differential anti-pathogen antigen activities

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Background: Intravenous immunoglobulin (IVIg) is derived from pooled plasma of thousands of healthy donors and contains immune and natural antibodies, representing the overall human IgG repertoire. Depending on the formulation, IVIg preparations contain variable amounts of monomeric and dimeric IgG. So far, little information is available on an eventual bias of Ab specificities within the monomeric and dimeric fractions.
Methods and results: In serological assays (ELISA), the dimeric fraction showed an increased recognition of a range of pathogen-associated protein antigens (Ags) 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. Cells were challenged with DT. Measurement of the proliferation of surviving cells showed equal neutralising capacities for both monomeric and dimeric fractions. However, quantification of the RSV neutralising activity revealed slightly increased anti-RSV activity within the dimeric IVIg fraction. In contrast, monomeric and dimeric fractions analysed for anti-Pseudomonas LPS, anti-Pneumococcus polysaccharide or anti-H. influenzae B polysaccharide activity by ELISA and showed a marked increased reactivity within the monomeric IVIg fraction. Dimer activity against pathogen-derived protein Ags showed a reduced activity compared to monomerised dimers, indicating blocking activity in the dimer fraction, assumed to represent anti-idiotype activity which is thought to play a role in immunoregulation by IVIg.
A test system for the anti- or pro-inflammatory activity of IVIg and its fractions (incl. sialylated vs. non-sialylated IVIg) is in development. Monocytes or monocyte-derived dendritic cells (MoDCs) are stimulated by immune complexes resulting in the expression of pro- and anti-inflammatory cytokines. Different IVIg preparations are comparatively analysed for their pro- or anti-inflammatory potential on both, protein and gene levels respectively.
Conclusions: In our study we saw clear differences in specificity among the IVIg fractions. However, functional differences and the specific contribution of the IVIg fractions to the effects of IVIg on the immune system (e.g. pro- vs. anti-inflammatory activities) are still unknown and need to be further investigated.

P166

Tertiary lymphoid organs develop a reticular cell network and a conduit system both of which are dependent on lymphoid tissue inducer cells

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Chronic inflammation often leads to the formation of so-called tertiary lymphoid organs (TLOs) resembling lymph nodes and Peyer's patches. This resemblance is striking for the presence of lymphoid cells, high endothelial venules and follicular dendritic cells but also for the characteristic localization of these cell types. So far, the stromal component of the lymphoid T zone within these infiltrates has been poorly defined. We investigated infiltrates in various types of transgenic or autoimmune-prone mice and found gp38-expressing fibroblastic reticular cells (FRC) being always associated with the T zone of larger infiltrates and often forming extensive networks. Similar to lymph nodes, they enwrapped extracellular matrix-filled conduits positive for endogenous CCL21 protein and fluorescent tracer. The maintenance of both FRC and conduits was largely lymphotoxin-alpha/beta dependent. We show that lymphotoxin-alpha/beta expressing lymphoid tissue inducer cells (LTIC) are present in TLOs, spleen and lymph nodes of adult RIP-CXCL13 transgenic mice. When crossed to mice deficient in the receptor retinoic acid receptor-related orphan-gamma (ROR-gamma), LTIC were absent from all organs investigated and TLOs failed to form. Therefore we propose that LTIC can play an active role in adulthood, such as in TLOs, and may participate in the formation and/or maintenance of stromal cells. Finally, the gp38-expressing FRC of the T zone are a characteristic feature of T cell rich infiltrates and are likely to contribute to the pathogenic immune response.

P167

Dendritic cell migration: the role of the adaptor protein SKAP-HOM

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SKAP55 (Scr kinase-associated phosphoprotein of 55 kD) and the related protein SKAP-HOM are adaptor molecules involved in inside-out signalling. In contrast to SKAP55, which is exclusively expressed in T cells, SKAP-HOM is an ubiquitous adaptor protein highly expressed also in dendritic cell (DC). Here, we show that the absence of SKAP-HOM does not affect the generation of DCs in the steady state as well as the organ colonization by DCs. In addition, the in vivo and in vitro maturation of DCs is also normal. In contrast, in FITC painting experiments, migration of DC from the skin to the draining lymph nodes is enhanced in the absence of SKAP-HOM. In vitro, the spontaneous and the CCL19-induced chemotaxis are increased in DC lacking SKAP-HOM, especially at low concentration of the chemokine. Moreover, the adhesion to extracellular matrix of mature but not immature DCs was found to be reduced in the absence of SKAP-HOM. In condition of low abundance of the antigen in vitro as well as in vivo, DCs lacking SKAP-HOM expression are less efficient in presenting the antigen and in inducing T cell stimulation. This is accompanied in vitro by a delay in the stabilization of the conjugates between DCs and T cells.
In conclusion, our data suggest that SKAP-HOM positively regulates adhesion to extracellular matrix as well as between different cell types, the latter process playing an important role during antigen presentation especially in the presence of low concentration of the antigen.

P168

Cross-priming versus direct priming in anti-tumoural immune responses

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Introduction: Cytotoxic T cells (CTLs) play a major role in protective immunity to tumors and may be induced by two possible pathways: by direct priming, where tumor cells directly present tumor antigens to naïve tumor-specific CTLs, or by cross-priming where professional antigen-presenting cells (pAPCs) cross-present captured tumor antigens to CTLs. However, the physiological relevance of these two pathways remains controversial.
Methods: In this study we examined cross-priming versus direct priming in the induction of anti-tumoral CTL responses after immunizing (H-2b x H-2d) F1 mice with different murine tumor cell lines all transfected with the glycoprotein (GP) or nucleoprotein (NP) of lymphocytic choriomeningitis virus (LCMV).
Results: Cross-priming was observed for the immunodominant epitopes LCMV-gp33 and -np118, but not for the subdominant gp283- or np396 epitopes, where specific CTLs were only induced when epitopes were presented directly on MHC I molecules of the immunizing tumor cell. Moreover, frequencies of CTLs specific for immunodominant epitopes were significantly higher after immunization with transfected tumors presenting the epitope on the corresponding haplotype compared to cross-priming. In this context, vaccination with syngenic tumor cells induced protective anti-tumoral immunity, whereas immunization with allogenic tumors only marginally delayed tumor growth.
Conclusion: Our results suggest that cross-priming may induce tumor-specific CTL responses to immunodominant epitopes but the induction of CTLs to subdominant epitopes was highly inefficient and direct presentation of these epitopes on MHC I molecules was necessary. Therefore, vaccination strategies using autologous or syngenic antigen-expressing cells are superior to immunization strategies based solely on cross-priming.

P169

Interactions of intraepithelial lymphocytes and intestinal epithelial cells in vitro

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In the normal intestinal epithelium approximately 10–15 intraepithelial T lymphocytes (IEL) per 100 epithelial cells are present. In the mouse, both TCRgamma delta and TCRalpha beta IEL are found. The TCRalpha beta IEL mostly consist of CD8alpha alpha and CD8alpha beta expressing T cells. In contrast to the cytotoxic CD8alpha beta T cells, CD8alpha alpha TCRalpha beta IEL are non-circulating, non-cytotoxic T cells expressing IL-10 and TGF-beta. Their expression of anti-inflammatory cytokines is indicative of a regulatory role in the intestine. However, the mutual interactions with epithelial cells and their functional consequences during physiological and inflammatory conditions are largely unknown. We established an in vitro co-culture system using the C57BL/6 derived intestinal epithelial cell lines mICcl2 and CMT93 and purified syngeneic IEL subsets to assess the effects of epithelial cell-IEL co-cultures on the integrity of the epithelial monolayer using transepithelial resistance measurements and paracellular tracer flux experiments. The presence of unstimulated IEL in the co-culture slightly increases the integrity of the epithelial monolayer whereas activated IEL and also activated CD8 splenic T cells disrupt the epithelial tight junctions, resulting in reduced transepithelial resistance. The disruption on the epithelial integrity is solely attributable to the CD8alpha beta IEL. The IEL-mediated disruption of the intestinal epithelium is strictly IFNgamma-dependent as activated CD8alpha beta IEL and CD8 TCRalpha beta splenocytes from IFNgamma KO mice do not disrupt the epithelial integrity. Currently, we are assessing the role of activated CD8alpha alpha TCRalpha beta IEL on the epithelial monolayer integrity and in particular, whether IL-10 and TGF-beta produced by CD8alpha alpha IEL may prevent IFNgamma-mediated effects such as chemokines secretion and proinflammatory markers upregulation in intestinal epithelial cells.

P170

Evaluation of the differential gene expression induced in macrophages infected with metastatic and non-metastatic Leishmania guyanensis parasites

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Introduction: Infections with L.guyanensis are distinguished by their ability to disseminate from the initial site of infection to the nasopharyngeal tissues forming destructive secondary lesions. Mucocutaneous Leishmaniasis (MCL) patients have a hyper-inflammatory response as shown by increased macrophage and T cell infiltration into the lesion, producing higher levels of IFNg, IL5, and TNFa and decreasing IL10R expression. It is known that the outcome of infection is influenced by both the host immune response and the infecting parasite. The factors involved in parasitic dissemination are poorly understood.
Methods: In the hamster model of L.guyanensis infection, different clones of the parasite were isolated and characterised as metastatic (M+) or non-metastatic (M-) from their ability to form secondary mucosal lesions. We used these M+ and M- disease causing parasites to infect macrophages and the differential gene expression was analysed using cDNA microarrays. Real-time PCR and protein analyses were used to confirm selected identified genes.
Results: Two chemokines important for a Th1 immune response were differentially expressed together with several cytokines including TNFa and IL6 in both C57BL/6 and BALB/c mouse macrophages. To further analyse gene regulation we infected macrophages from MyD88-/- and TRIF-/- mice with the M+ and M- parasites. We found that the loss of TRIF abrogated gene expression at the RNA and protein level. The role of individual TLRs in the macrophage response to infection with M+ parasites is currently being investigated.
Conclusion: This study has shown that the host response can be differentially modulated depending on the infecting parasite. Several genes and pathways were identified that could be involved in promoting development of MCL. This is very attractive for future studies as a greater understanding of the mechanism of parasitic dissemination could provide insights for new MCL therapies.

P171

Relevance of atopy patch testing with cockroach allergen in patients with atopic dermatitis

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Background: Allergens from house dust mites represent an important trigger in a subgroup of patients with atopic dermatitis (AD). It is thought that house dust mites and cockroaches contain some crossreactive allergens such as tropomyosin.
Objective: The aim of this study was to investigate whether cockroach allergens may elicit eczematous skin test reactions and to analyze the relationship between cockroach and house-dust mite sensitivity in subjects with AD.
Methods: Twenty-three patients with AD and 9 non-atopic controls

were recruited in the Out-patient Clinic for Allergology, University of Bern. Atopy patch tests (APT) were performed with biologically standardized (200 IR/g) cockroach and house-dust mite extracts (Stallergènes, France) and evaluated after 48 and 72 hours. In addition, skin prick tests (SPT) with a panel of common inhalant allergens, total serum IgE, and specific IgE tests for cockroach and house dust mites were performed.
Resultats: A positive APT reaction to cockroach was found in 10/23 (43%) patients with AD, a positive SPT in 6/23 (26%) patients. Twelve of the 23 (52%) patients with AD were also sensitized to house-dust mites. Positive APT reactions for cockroach showed no significant correlation with the SPT and specific IgE levels for this allergen. There was no significant correlation between the positive SPT, APT and specific IgE to cockroach and house dust mite. No positive test reactions were observed in the non-atopic controls.
Conclusion: Our results suggest that cockroach allergens can trigger eczematous skin test reactions in a substantial number of patients with AD. There was no significant relationship between cockroach and house-dust mite sensitivity detected.

P172

Dextran sulfate attenuates cold ischaemia-induced innate immune response and facilitates RIB 5/2-induced long-term allograft survival

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Ischemia/reperfusion (I/R) injury of a graft leads to an increased activation of the innate immune response of the recipient. This boost of anti-graft immune response may be the reason for failure of immunological tolerance induction in the context of prolonged graft ischemia times. Here we show that treatment with the endothelial cell (EC) protectant dextran sulfate (DXS, MW 5000) restores a quiescent and 'non-dangerous' state of the graft and facilitates long-term survival.
Heterotopic heart transplantation from DA to Lewis rats was carried out and long-term graft survival induced by non-depleting anti-CD4 monoclonal antibody (RIB 5/2) with or without DXS. Cold graft ischemia was either 20 min or 12 h. Median survival times (MST) of RIB 5/2 only or RIB 5/2+DXS treated recipients in the 20 min ischemia group were 86 and 81 days, respectively, with >30% of long-term graft survival (>175 d) in both groups. In the 12 h ischemia group RIB 5/2 only treatment led to chronic rejection (MST = 49.5 d). In contrast, treatment with RIB 5/2+DXS induced long-term graft survival (MST = 100d, $p < 0.05$). Analysis of grafts subjected to 12 h of cold ischemia at 1d post transplantation revealed that RIB 5/2+DXS treatment significantly reduced EC activation, deposition of complement as well as infiltration of granulocytes and monocytes/macrophages. In the long-term surviving allografts of RIB 5/2+DXS treated recipients an upregulation of the tolerance associated gene 1 (TOAG-1) was found by RT-PCR.
In summary, our results suggest that DXS attenuates acute graft injury related to prolonged ischemia time and facilitates long-term graft survival.

P173

The 3-dimensional structure of secretory component reveals the implication of surface glycans crucial for its microbial scavenger properties

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Background: Human polymeric Ig receptor (pIgR) allows the transport of polymeric IgA (pIgA) to mucosal surfaces where it is released as secretory IgA (SIgA), i.e. pIgA linked to the portion of pIgR named secretory component (SC). SC, composed of five glycosylated Ig-like domains, ensures stability and correct localization of SIgA, which exerts its neutralizing properties at mucosal surfaces. Furthermore, SC can be found free in secretions and has been shown to have scavenger properties toward various mucosal pathogens.
Methods: Complementary biochemical analysis were used to identify essential structures on SC, which might confer protective effects against mucosal pathogens. In addition, the 3-dimensional structure of SC was obtained using a combination of techniques including X-ray and neutron scattering, analytical ultracentirfugation and computer modeling.
Results: Here we show that SC binds to Clostridium difficile toxin A and to Escherichia coli adhesion molecule intimin, and that through this interaction target cells are protected from infection. More precisely, binding between pathogen-derived structures and SC is abolished when SC is deglycosylated or denaturated, meaning that sugars, together with the overall conformation of SC are crucial for protection.
In addition to these novel biological data, the solution structure of free SC was determined for the first time. SC adopts a J-shaped structure as shown in figure 1. Sugar residues are clustered in two main regions, and thus may favor a good anchoring of pathogenic structures. Importantly as well, the known IgA-binding motifs on SC are unmasked and thus remain free to interact with pIgA.
Conclusion: These data further confirm the scavenger properties of free SC toward mucosal pathogens and identify oligosaccharides as binding sites for various bacterial structures. In the context of SIgA, glycans are on the surface of the complex, and can constitute further unspecific-binding sites, thus conferring to the molecule properties associating innate and adaptive immunity.

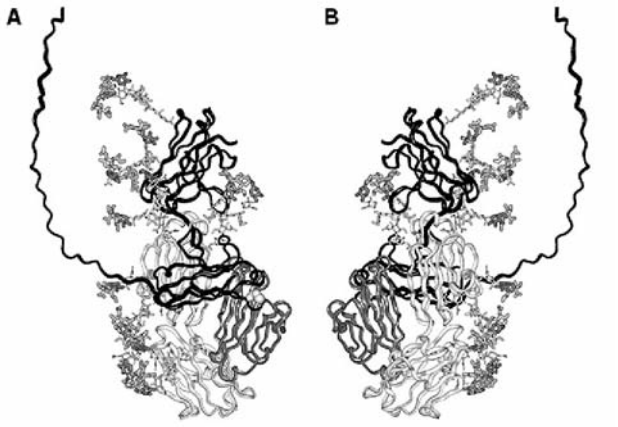

Figure 1: Solution structure of human secretory component.
The best-fit model of free secretory component is shown in **A**, and rotated by 180° in **B**. The 5 Ig-like domains are represented with their carbohydrates at the surface of the molecule.

P174

Overlapping functions of IL-7 and TSLP in lymphoid development

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Interleukin 7 (IL-7) and IL-7 receptor (IL-7R) deficient mice have severe defects in lymphopoiesis and in the development of lymph nodes and Peyer's patches. Originally discovered for its ability to sustain B cell development in vitro, thymic stromal lymphopoietin (TSLP) is a cytokine which binds to the IL-7R alpha chain. We tested if increased TSLP availability through transgene (Tg) expression could restore lymphopoiesis in IL-7-/- mice. Indeed, we found that TSLP Tg expression rescues all stages of B cell development, increases thymic and splenic cellularities, restores double negative thymocyte populations, gamma delta T cell generation and peripheral B and T cell compartments. Lymph node and Peyer's patch numbers are normal in TSLP Tg IL-7-/- mice. Bone marrow chimeras demonstrate that hematopoietic progenitor cells from adult wild type mice efficiently differentiate towards B and T cell lineages in lethally irradiated IL-7-/- mice provided TSLP Tg is expressed in these mice. In vitro, TSLP promotes the differentiation of uncommitted adult bone marrow progenitors towards B and T lineages and the further differentiation of DN1 and DN2 thymocytes. Altogether, our results show that TSLP has a striking capacity to promote lymphoid development.

P175

Regulation of intestinal glucocorticoid synthesis in experimental colitis

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Glucocorticoids (GC) are important members of steroid hormones with widespread activities in metabolism, development and immune regulation. The adrenal glands are the major source of GC and

release these hormones in response to psychological and immunological stress. However there is increasing evidence for extra-adrenal GC synthesis in thymus, brain, skin and vascular endothelium. We recently described that the intestinal mucosa expresses steroidogenic enzymes and is an important source of GC. Intestinal GC critically regulate local immune homeostasis, as in the absence of intestinal GC T cell activation is impaired. Interestingly, injection of anti-CD3 antibodies or viral infection critically induce GC in epithelial cells, which have in turn a strong counter-regulatory activity on the activation of local immune cells.
The aim of the present study was to elucidate the role and regulation of intestinal synthesized GC during the various stages of the pathogenesis of inflammatory bowel disease (IBD). For this purpose we induced colitis in mice using three well described protocols of IBD, the dextran sulfate sodium-model (DSS), the trinitrobenzen-sulphonic acid-model (TNBS) and the oxazolone model. In DSS- and TNBS-, but not in oxazolone-induced colitis, we observed a transient up-regulation of steroidogenic enzymes as well as a transient increase in GC synthesis in large bowels of diseased animals compared to controls. Interestingly, the observed decrease in GC synthesis correlates with an increase in clinical and histopathological parameters.
In conclusion, we showed that the intestinal mucosa is capable of synthesizing and releasing considerable amounts of GCs upon induction of DSS- and TNBS-induced colitis, most likely to regulate the activity of overwhelming immune effector cells during inflammation. Intestinal GC synthesis might slow down the local colonic inflammation but is not sufficient to block experimentally-induced colitis.

P176

Granulocyte-macrophage colony-stimulating-factor recruits distinct dendritic cell subsets into the central nervous system of mice and promotes experimental autoimmune encephalomyelitis

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Dendritic cells (DC) are a well defined subpopulation of antigen presenting cells (APC). 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). It has been shown that the growth factor granulocyte-macrophage colony-stimulating factor (GM-CSF) preferentially induces the expansion of myeloid DCs (CD45hi CD11c+ CD11b+). This DC subset can be further differentiated into a F4/80-negative and a F4/80-positive fraction. We previously found F4/80- DCs within the EAE-diseased central nervous system (CNS) to be stimulatory and F4/80+ DCs to be inhibitory. Thus we aimed at defining the role of GM-CSF in the recruitment of either stimulatory or inhibitory CNS-associated DCs in vivo during EAE. Mice treated with GM-CSF developed more severe EAE accompanied by a stronger increase in the number of DCs. Complementarily, mice lacking the GM-CSF receptor did not develop EAE and did not contain any infiltrates within the CNS during disease development. In order to differentiate between bone marrow (BM)-derived and CNS-derived APCs, we generated BM-chimeric (CD45.1 -> CD45.2) animals. The treatment with GM-CSF resulted in a preferential induction of F4/80- BM-derived DCs. In contrast, CNS-derived DCs were predominantly of the F4/80+ phenotype. In summary, the data suggest that distinct DC-subpopulations are generated in the CNS of EAE diseased animals. Both the DC recruitment and the disease severity of EAE are enhanced in the presence of GM-CSF. The chimera experiment further suggest that the effect of GM-CSF on stimulatory F4/80- BM-derived DCs dominates over the GM-CSF-mediated induction of F4/80+ CNS-derived DCs shown to be inhibitory.

P177

Degranulation of preformed Fas (CD95) ligand in activated T-cells

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Fas ligand (FasL, CD95L) is a member of the Tumor Necrosis Factor family and a potent inducer of apoptosis. It is mainly expressed on activated T cells and NK cells, where it can mediate target cell killing by engaging its receptor Fas. The expression of FasL is tightly regulated by both, transcriptional events as well as post-transcriptional mechanisms. In particular, activated T cells store preformed FasL in secretory vesicles and release it to the cytoplasmic membrane upon restimulation. The aim of this study is to investigate the biochemical processes leading to subcellular redistribution of FasL upon T cell activation.
Restimulation of murine T cell blasts with immobilized anti-CD3 antibody or PMA/Ionomycin results in functional FasL cell surface expression by de novo protein synthesis and the release of preformed FasL from secretory vesicles (degranulation). In contrast, stimulation of T cell blasts with the phorbol ester PMA alone results in FasL cell surface expression, which is solely dependent on FasL degranulation. Stimulation with the calcium ionophore Ionomycin alone was not sufficient to induce FasL degranulation. PMA induced degranulation is protein kinase dependent and mediated by actin filament dependent intracellular transportation system. Surprisingly, an involvement of PMA activated protein kinase C and protein kinase A isoforms could be excluded. Although FasL partially colocalizes with the secretory lysosomal marker CD63, PMA only induced cell surface expression of FasL but not CD63. This suggests that the degranulation of different granular proteins is regulated by distinct biochemical pathways. Future studies aim at investigating the differential subcellular transportation of FasL versus other granular proteins, i.e. perforin, granzyme B, lamp1 and CD63 in different T cell subsets. The understanding of the degranulation mechanisms of cytotoxic T cell effector molecules may give important insight into the differential use of perforin versus death ligand mediated killing.

P178

A new animal model to study the requirements for the induction and tolerance mechanisms in experimental induced autoimmune myocarditis

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Introduction: Severe inflammation of the heart muscle induced by cardiotropic pathogens may broaden the immune response to heart autoantigens, like the abundantly expressed myosin heavy chain alpha (myhca). Although the pathogen might be cleared a chronic autoimmune response may lead to dilative cardiomyopathy (DCM), which represents a prevalent cause of human heart disease and heart failure. The induction of a myosin specific immune response could be uncoupled from infection by using the myhca-derived peptide (amino acids 614–629). Immunizing BALB/c mice with this peptide induces a strong CD4 T cell response leading to autoimmune myocarditis.
Method: In order to further characterize the mechanisms and effector molecules involved in autoimmune myocarditis and to evaluate therapeutic strategies, we have generated a novel transgenic mouse model. In this model (ROSA-IM mouse) the CLIP fragment of an additional invariant chain (Ii) is replaced by the myhca-peptide (614–629) and is placed into the ROSA-26 locus by homologous recombination. Principally a stop cassette flanked by loxP sites inhibits peptide expression, but expression is achieved by the presence of Cre-recombinase.
Results: Mice expressing the myhca-peptide (614–629) on all antigen presenting cells (APC) show some residual CD4 response after peptide immunization compared to control mice but were completely protected from myocarditis.
Conclusion: The ROSA-IM mouse represents therefore a versatile tool to dissect the factors involved in activation and tolerization of myhca-specific CD4 T cells, to study the role of the different APC for tolerance induction and to investigate the immunohierarchy of the myhca-peptide epitope in the context of heart myosin.

P179

The hepatocyte growth factor (HGF) receptor c-met is expressed by macrophages in the CNS during the peak phase of experimental autoimmune encephalomyelitis (EAE)

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In EAE, a well-established animal model of multiple sclerosis, demyelination is induced by myelin-specific T-cells, antibodies and activated macrophages/microglia. Recovery from EAE is initiated by Th2 cytokines, such as TGF-beta, which suppress T-cell expansion and the production of myelin-toxic molecules by macrophages. In an earlier report we described that microglia, which are the principal CNS-resident immune cells, release HGF upon TGF-beta treatment in vitro, suggesting a potential role for HGF in EAE.
Using immunohistochemistry, we now show that expression of the

HGF receptor, c-met, is restricted to the peak phase of EAE disease and is mainly expressed by CD11b+ macrophages/microglia within demyelinated lesions in the spinal cord. In addition, a small percentage of CD11c+ dendritic cells (DCs) and NG2+ OPCs were found to express c-met, whereas CD4+ T-cells were completely c-met negative. Flowcytometric analysis confirmed these observations. The timing of c-met expression, with expression limited to the peak phase of disease, and the robust expression by macrophages indicates a potential pro-inflammatory role for the HGF-c-met pathway in EAE.
Subsequent in vitro experiments, using bone marrow-derived macrophages (BMM), showed that quiescent macrophages do not express c-met but that c-met mRNA and protein expression can be induced by treatment with TNF-alpha or LPS. Furthermore, experiments with soluble TNF receptor and monoclonal anti-TNF-alpha antibodies demonstrated that LPS-induced c-met expression is mediated via autocrine TNF-alpha signalling.
Although the function of c-met in macrophages remains unclear our data suggests it plays no role in classical macrophage functions such as phagocytosis, cytokine secretion and NO production. Currently, EAE experiments with macrophage-specific c-met knockout mice as well as gene chip arrays with HGF-treated BMM are being performed to elucidate the role of c-met in macrophages in EAE.

P180

The role of the pro-apoptotic Bcl-2 family member Bim in GM-CSF-regulated neutrophil apoptosis

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Background: Neutrophils are constantly produced in large numbers in the bone marrow. After maturation, they enter into the circulation and undergo apoptosis. However, under inflammatory conditions, neutrophil apoptosis is delayed due to survival factor exposure, and, consequently, cell numbers increase. An important pro-inflammatory cytokine involved in the regulation of neutrophil survival/activation is granulocyte-macrophage colony stimulating factor (GM-CSF). Although GM-CSF mediates anti-apoptotic effects in neutrophils, it does not prevent apoptosis, and the survival effect is both time-dependent and limited. We therefore hypothesised that GM-CSF, in spite of mediating anti-apoptotic effects, allows the activation of pro-apoptotic pathways.
Methods: Global gene expression was assessed using oligonucleotide microarrays. For protein detection cell lysates were prepared and Bim protein expression was assessed using immunoblotting. Cell survival was determined by flow cytometric analysis of ethidium bromide uptake.
Results: Analyzing global gene expression in untreated and GM-CSF – stimulated human peripheral blood neutrophils revealed that GM-CSF induced the expression of pro-apoptotic Bcl-2 family member Bim. To verify these findings, we performed immunoblotting analysis and observed increased Bim protein levels in GM-CSF – stimulated blood neutrophils compared to freshly isolated or cultured control blood neutrophils. Bim upregulation was dependent on de novo transcription and translation as it was blocked by both cycloheximide (translation inhibitor) and actinomycin D (transcription inhibitor). Pharmacological inhibition of PI3K (LY294002) blocked GM-CSF – mediated survival and, surprisingly, decreased Bim expression in neutrophils. The functional role of Bim was investigated using Bim deficient mouse neutrophils. Lack of Bim expression reduced spontaneous neutrophil death. Moreover, in the absence of Bim, both GM-CSF and IL-3 demonstrated much higher efficacy to block neutrophil apoptosis.
Conclusions: These data demonstrate a functional role for Bim in the regulation of neutrophil apoptosis and suggest that GM-CSF and other neutrophil hematopoietins initiate a pro-apoptotic counter regulation that involves up-regulation of Bim.

P181

TGFb receptor II gene deletion in leukocytes exacerbates experimental autoimmune encephalomyelitis

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The TGFb family of proteins are secreted molecules with potent immunoregulatory properties. All three isoforms, TGFb -1, -2 or -3, signal via the same heteromeric receptor complex, consisting of a ligand binding TGFb receptor type II (TRII) and a TGFb receptor type I. TGFb -1 secreted by macrophages and monocytes can regulate many activities of these immune cells such as expression of MHCII, chemotaxis, production of cytokines and their receptors, production of reactive oxygen species and nitric oxide. Activation and infiltration of macrophages is seen in various disease models including experimental autoimmune encephalomyelitis (EAE). The current study investigates the role of TGFb on the macrophage compartment in inflammatory and autoimmune disease models. In order to interrupt the TGFb-signaling pathway in macrophages, we delete the TRII by breeding floxed TRII mice with mice expressing Cre recombinase under the macrophage-specific mouse lysozymeM promoter. The LPS-induced production of TNFa is not inhibited by TGFb in Cre-expressing macrophages. Macrophages of wildtype animals show a 50–80% reduction of TNFa secretion in the presence of TGFb. The results show clearly that the TGFb-signaling pathway in macrophages is not functional. TGFb macrophage receptor knockout mice immunized with MOG35-55 to develop EAE show a significantly higher disease score in the late phase of the disease compared to control animals. Mononuclear cells isolated from the CNS of EAE mice are currently tested for cytokine production.

P182

Activation of NK cells in drug hypersensitivity

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Introduction: Cytotoxic cells are critically involved in different forms of drug hypersensitivity. We have recently shown that drug-specific cytotoxic T cells can be detected in peripheral blood of drug-allergic patients. The role of natural killer (NK) cells, as an important component of innate immunity, in drug induced cytotoxicity has not been investigated so far.
Methods: To asses whether specific drug stimulation can induce activation and degranulation of NK cell in peripheral blood of drug allergic patients, peripheral blood mononuclear cells (PBMC) of drug allergic patients and healthy subjects were isolated from blood and frozen. After thawing, PBMC were incubated with medium alone (negative control), with the culprit drug and superantigen SEB (positive controls). Following stimulation, three parameters were evaluated: a) cell surface expression of CD69 as a stimulation marker and b) CD107a as a degranulation marker by FACS, and c) measurement of granzymeB production by ELISPOT.
Results: Stimulation of PBMC from allergic patients with the specific drug lead to the upregulation of CD69 and CD107a on the surface of NK cells. Delayed upregulation of CD107a (the earliest after 24h) on NK cells from freshly isolated PBMC correlated with the kinetics of CD107a expression on CD4 and CD8 T cells. Interestingly, the percentage of NK cells upregulating CD69 and CD107a after stimulation with the drug was 10–20 times higher than the percentage of activated T cells, and was thus a more robust marker for drug sensitization than the activation of T cells itself. Within total PBMC, the number of cells releasing granzymeB was much lower and corresponded better to the number of CD107a-positive T cells, however. Restimulation after a 7d culture with the drug and antigen presenting cells caused immediate (5h) degranulation of T cells but not of NK cells.
Conclusions: Activation of peripheral blood cells of drug allergic patient by drugs leads to activation of NK cells. This activation is probably due the cytokines released by drug specific T cells and is not able to cause a granzymeB-dependent cytolytic activity of NK cells. Since the reaction of NK cells is stronger than the reaction of specific T cells, monitoring of NK cell activation may be advantageous in in vitro testing of drug hypersensitivity.

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Towards improvement of structural and functional basis of TCR avidity for tumour antigen

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Background: CD8+ T lymphocytes recognize and destroy virus-infected or tumor cells. However, the tumor-specific cytotolytic T lymphocytes often fail to mediate a clinically effective immune response. A major reason might be the relative lack of high avidity

T cell receptors (TCR) within the pool of tumor-specific T cells. It is therefore tempting to reengineer the TCR sequence to increase its affinity for common tumor epitopes. As proof of concept, we have chosen to study the HLA-A2/NY-ESO-1 model that provides an ideal system to study the effect of TCR point mutations on CD8+ T cells.
Methods: Using a rational in silico approach in which the TCR sequence is optimized in a predictive and controlled manner, optimized TCR candidates are tested in redirected TCR gene transfer experiments via lentiviral transduction. Results of the MM-GBSA calculations allowed the identification of 2 single and 1 double amino acid replacements for a dominant TCR-BV13 clonotype located within the CDR2beta region, designed to either increase or decrease its affinity for A2/NY-ESO-1.
Results: Cell surface expression of TCR-BV13 was very similar among bulk CD8+ T cells transduced with the different lentiviral supernatants (wt, mutants A-C). In contrast, the proportion of multimer-stained cells transduced with TCR-mutant A, predicted to have a decreased affinity for the pMHC complex, was significantly lower than the proportion of stained cells expressing wt TCR, and mutant B and C TCR, predicted to increase their affinity for pMHC. We next measured TCR-pMHC off-rates using multimer decay assays, and observed that the TCR/multimer off-rate for mutant A was much faster and in sharp contrast to the kinetics observed for mutant C, indicating that mutant A formed TCR/multimer complexes of relatively low stability. However, despite our data showing that mutant C formed TCR/multimer complexes of relatively high stability, there was no significant advantage for the CD8+ T cells expressing mutant C in the killing of tumor cell lines.
Conclusion: Combined bio-modeling and functional experiments allowed for the first time to specifically assess the impact of single amino acid replacements within the TCR CDR2beta region on both TCR-pMHC affinity and functional avidity. Ongoing experiments assessing down-stream TCR signaling and survival pathways on these transduced T cells should further enhance our understanding of the structural and functional basis of TCR recognition for the NY-ESO antigen.

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Crosstalk between human mesenchymal stromal cells and allogenic natural killer cells

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Background: Mesenchymal multipotent stromal cells (MSC) are post-natal stem cells that can inhibit steroid-resistant graft-versus-host-disease (GVHD) occurring after allogenic hematopoietic stem cells transplant. Natural killer cells (NK), infused once GVHD has subsided, can eradicate the eventual leukemia relapse provoked by immune suppression. So far the interplay between MSC and NK is not fully understood. For this reason, we investigated the interactions between MSC and NK in vitro.
Methods: NK were purified from buffy coat by Ficoll-Hypaque gradient centrifugation and negative selection using immuno-labeled magnetic beads. MSC were isolated from femur heads of patients who underwent hip replacement surgery. CFSE-labeled NK were cultured in a medium supplemented with 5% platelet lysate with IL-15 (0–25 ng/ml) in absence or presence of MSC. After 6 days NK proliferation was assessed by CFSE dilution. NK cytotoxicity was tested using a flow cytometry based assay.
Results: Optimal proliferation of NK was achieved using 25 ng/ml of IL-15 (87% CFSE low cells). This value was reduced to 40% in presence of MSC ($n = 5$, $p = 0.008$, Mann-Whitney). However in these conditions, NK were cytotoxic towards the MSC stroma. By contrast, preliminary experiments ($n = 2$) performed under conditions that sustained NK survival but not proliferation in absence of MSC, showed that NK proliferation could be triggered by MSC. This suggested that MSC could also have a proliferative effect on NK.
Conclusion: These in vitro data indicate that the effect of MSC on NK correlates with the activation status of the later, a fact that is consistent with in vivo observations on leukemic patients. Infused MSC inhibit acute GVHD but are rarely detected in vivo, suggesting that NK initially co-injected with the hematopoietic stem cells, optimally activated by the GVHD possibly kill them. By contrast delayed allogenic NK infusion performed after GVHD has settled down, efficiently eradicates relapsing leukemia without inducing generalized inflammation or impairing hematopoiesis. As hematopoiesis depends on functional host MSC, this suggests that when inflammation is low, NK do not kill host MSC. Moreover NK are not inhibited by MSC as they can effectively eradicate the residual leukemic cells. Further understanding the crosstalk between MSC and NK has to be achieved before cell therapies involving both cell types can be envisaged.

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Manipulation of MHC class I antigen presentation by an LMP7-specific inhibitor

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The proteasome is responsible for the generation of most epitopes presented on MHC class I molecules. Treatment of cells with interferon-gamma leads to the replacement of the constitutive catalytic subunits beta1, beta2 and beta5 by the inducible subunits LMP2, MECL-1, and LMP7. The incorporation of these subunits seems to be required for the production of several MHC class I restricted T cell epitopes. In this study, we investigated the effect of a novel LMP7-specific inhibitor on antigen presentation. Fluorogenic assays with substrates specific for the chymotrypsin-like activity demonstrated that the inhibitor acts specifically on LMP7 in a concentration range of 100–300 nm. Furthermore, experiments with immunoproteasome deficient mice revealed that the generation of the male HY-derived Uty246-254 epitope is not only LMP7 dependent, as previously reported, but also dependent on LMP2. Treatment of male splenocytes with the LMP7-specific inhibitor reduced the Uty246-254 presentation to background levels. Flow cytometric analysis of inhibitor treated splenocytes showed a reduction of MHC-I expression on wild type, but not on LMP7-deficient cells. Moreover, inhibition of LMP7 in cells infected with lymphocytic choriomeningitis virus (LCMV), led to decreased presentation of the LCMV-derived GP33-41 epitope. In vivo experiments demonstrated that the Uty246-254 presentation and the CD8+ T cell response to Uty246-254 is dramatically reduced in LMP7-inhibitor treated mice. Taken together, we were able to show for the first time, that a specific inhibition of the IFN-gamma inducible immunoproteasome subunit LMP7 leads to a reduced presentation of several MHC class I restricted epitopes in vitro and in vivo.

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The effect of CD44 ligation on granulocyte cell death

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Background: CD44 is a transmembrane glycoprotein involved in cell-cell and cell-matrix interactions. It is expressed in multiple cell types and implicated in a wide range of physiological and pathological processes, such as morphogenesis, organogenesis, hematopoesis, leukocyte activation, lymphocyte homing, wound healing, cell migration, tumor growth, metastasis and cell death.
Initial studies on neutrophils suggested that GM-CSF increases the CD44 mRNA level. Since ligation of CD44 with the monoclonal antibody A3D8 was shown to induce death in leukemia cells, we became interested to investigate, whether CD44 induces cell death in granulocytes.
Methods: Cell death was assessed by ethidium bromide uptake and flow cytometric analysis. To determine whether cell death is due to apoptosis, redistribution of phosphatidylserine and oligonucleosomal DNA fragmentation (in the presence of propidium iodide) were measured by flow cytometry.
Results: CD44 ligation enhances spontaneous neutrophil cell death after GM-CSF priming in a concentration-dependent manner. Mimicked proinflammation by GM-CSF and fMLP enhanced CD44-mediated neutrophil cell death, while other cytokines like G-CSF, IL-8, IFN-alpha and IFN-gamma were unable to increase CD44-mediated neutrophil cell death.
In the presence of GM-CSF, CD44 ligation induced phosphatidyl-serine redistribution and DNA fragmentation, indicating that mature neutrophils are dying by apoptosis.
In addition, morphological investigations of GM-CSF primed and CD44-ligated neutrophils demonstrated aberrant cytoplasmic vacuole formations.
Similarly to neutrophils, CD44 ligation enhanced eosinophil cell death after GM-CSF priming. In patients suffering from hypereosinophilic syndromes, additional priming with GM-CSF had no further effect on eosinophil cell death, indicating that these eosinophils were already primed.
Conclusions: CD44 ligation initiates both apoptotic and nonapoptotic pathways in human granulocytes, which may promote the resolution of inflammatory responses.

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Cathepsin D initiates neutrophil apoptosis and its inhibition blocks the resolution of inflammation

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Background: Although the lysosomal cathepsins have often been considered as intracellular proteases able to mediate caspase-independent death, there is evidence that they act in concert with caspases in apoptotic cell death. In particular, the cysteine protease cathepsin B and the aspartic protease cathepsin D have been reported to be involved in apoptosis regulation. Since neutrophils rapidly undergo apoptosis following phagocytosis of bacteria, we hypothesized that azurophilic granules, in which cathepsins are located and intracellular bacterial killing occurs, might be able to somehow trigger the normal apoptotic program in these cells.
Methods: To resolve the issue of whether cathepsins are involved in neutrophil apoptosis pathways, we specifically inactivated cathepsin B and D, respectively, by both genetic and pharmacological means. Neutrophil death was assessed by uptake of ethidium bromide and flow cytometric analysis. To determine whether cell death was apoptosis, redistribution of phosphatidylserine in the presence of propidium iodide and oligonucleosomal DNA fragmentation were measured by flow cytometry. Immunofluorescence analysis of freshly isolated or cultured neutrophils was performed to follow time-dependently the release of cathepsin D from azurophilic granules into cytosol. Subsequent activation of caspase-8 and caspase-3 by cathepsin D was analyzed either by cell-free assay followed by western blot analysis or by enzymatic assay.
Results: We report here a new pro-apoptotic pathway in which cathepsin D directly activates caspase-8. Cathepsin D is released from azurophilic granules in neutrophils in a caspase-independent manner. Under inflammatory conditions, however, the translocation of cathepsin D in the cytosol is blocked. Pharmacological or genetic inhibition of cathepsin D resulted in delayed caspase activation and reduced neutrophil apoptosis. Cathepsin D deficiency or lack of its translocation in the cytosol prolongs innate immune responses in experimental bacterial infection and in septic shock.
Conclusion: Thus, we identified a new function of azurophilic granules, which regulate, besides their role in bacterial defense mechanisms, the life-span of neutrophils and therefore the duration of innate immune responses through the release of cathepsin D.

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Acute physical exercise affects natural killer cell numbers and function

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Introduction: Psychological and chronic physical stress increase the number of natural killer (NK) cells in the circulation, but decrease NK cell cytotoxicity. Since the influence of acute physical exercise (APE) on PBMC and NK cells remains unclear, we analyzed kinetics, phenotype and NK cell function before and immediately after short intensive exercise.
Material and methods: Heparinized peripheral blood (50 ml) was obtained from 5 healthy volunteers immediately before and after running up and down 6 flights of stairs. The phenotype of PBMC and NK cells, including the NK activating receptors NKp30, NKp44, NKp46, and NKG2D, was analyzed by flow cytometry. The percentage of IFN-g secreting NK cells was determined by a capture assay after 4 and 24 h, total IFN-g release by ELISA after 48 h of incubation with either low (50 U/ml) or high (500 U/ml) doses of IL-2.
Results: APE led to an increased number of PBMC (x 1.2 to 1.5) and NK cells (x 2.1 to 6.0) in all 5 donors. A 40% decrease in the percentage of CD56bright NK cells and a slightly decreased expression of NKG2D and NKp46 (preliminary results) was noted after APE. In contrast, APE did not influence the basal percentage of IFN-g secreting NK cells. After 4 h of IL-2 activation we found a 2-3 fold increase in the percentage of IFN-g secreting NK cells (n = 3), whereas a reduction of 60–70% (±15%) was detected after 24h (n = 5). After 48 h, IFN-g release was reduced to 50% (low dose IL-2) and 34% (high dose IL-2).
Conclusions: In summary, APE had a profound effect on circulating NK cell numbers (2–6 fold increase) and function (decreased percentage of IFN-g secreting NK cells after a 24h and decreased IFN-g accumulation after 48h of IL-2 activation). Therefore, if running is used to increase the numbers of available human NK cells for ex vivo experiments, one should be aware of the functional changes induced by APE and collection conditions should be as uniform as possible to exclude potential interferences on the experimental results.

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CD8 T cell differentiation and TCR repertoire dynamics in Epstein-Barr and cytomegalovirus infected individuals

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Protective immune responses against viral infections involve the selection and generation of differentiated antigen-specific CD8 T lymphocytes with potent effector functions, restricted clonal diversity and increased avidity of T cell receptors (TCR). Our objective is to identify correlates of immune protection in humans by analyzing the highly efficient responses to chronic viral infections. We analyzed the immune responses against chronic Epstein-Barr (EBV) and cytomegalovirus (CMV) infections in healthy individuals by determining the phenotype and repertoire characteristics of viral specific T cells. We found that EBV-specific CD8 T lymphocytes consist primarily of early-differentiated effector memory cells (EM/CD28+), while CMV-specific T lymphocytes are mostly composed of differentiated effector cells (EM and EMRA/CD28-). Although the proportions of these distinct T cell subsets were different among EBV- and CMV-specific T cells, ex-vivo analysis of several functionally relevant proteins (CD27, CD57, CD127, granzyme B, perforin) revealed that they follow the same pathway of T cell differentiation, from EM/CD28+ to EMRA/CD28-. Ex-vivo TCR repertoire analysis showed that the EBV specific responses are dominated by several T cell clonotypes bearing the BV2 and BV4 chains, while CMV responses are highly restricted to few clonotypes, but with BV chains that can vary from donor to donor (BV13, BV14, BV15). In order to determine the frequency of each clonotype and study the dynamics of dominance along the pathway of differentiation, we generated EBV and CMV specific T cell clones from the least differentiated and from the most differentiated subsets. In vitro TCR repertoire analysis showed that while certain clonotypes were selected, other clonotypes were lost with differentiation and were most dominant in the early-differentiated compartment. We are currently investigating factors such as TCR affinity that may be involved in the selection of particular clonotypes towards differentiation. We conclude that immune responses to chronic infections are unique to each virus, although they consist of antigen primed and functionally active effector memory T cells. Altogether, combined analysis of T cell differentiation and TCR repertoire will provide novel insights in the dynamics of T cell responses, and the identification of correlates of immune protection will be crucial for the development of therapeutic vaccination against chronic viral infections and cancer.

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Eosinophils release mitochondrial DNA to trap and kill bacteria

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Although eosinophils are considered as being useful in defense mechanisms against parasites, their exact function(s) in innate immunity, however, remains unclear. The aim of this study was to better understand the role of eosinophils infiltrating the gastrointestinal tract. We show here that lipopolysaccharide (LPS) from gram-negative bacteria activates interlukin (IL)-5 primed eosinophils to release mitochondrial DNA and granule proteins in a reactive oxygen species (ROS) dependent manner, but independent from eosinophil death. The mitochondrial DNA and the granule proteins from extracellular structures able to bind and kill bacteria both in vitro and under inflammatory conditions in vivo. Therefore, eosinophils contribute to innate immune responses by fighting against bacteria in the extracellular space using mitochondrial DNA. This newly identified function of eosinophils might represent an important defense mechanism to protect the host from uncontrolled invasion of bacteria in inflammatory bowel diseases associated with epithelial cell damage.

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The chemokine CXCL14/BRAK has antimicrobial activity

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Several chemokines possess potent killing activity against bacteria and fungi, similar to antimicrobial peptides such as defensins and cathelicidins. The antimicrobial activity of chemokines is explained by their high cationicity and the topological formation of large, positive electrostatic patches on the surface of the molecule, features they share with defensins and which allow electrostatic interaction with the anionic moieties on the surface of bacteria thereby disrupting the integrity of their membrane. The chemokine CXCL14/BRAK is constitutively expressed in healthy skin, notably in keratinocytes and dermal fibroblasts, but also in other epithelial tissues. Its function, however, is poorly characterized and its receptor still unknown. Due to its prominent expression at physical barriers, we hypothesized CXCL14/BRAK to be a candidate among the chemokines to act as an antimicrobial peptide.
We examined gram-negative and gram-positive bacteria and the fungus Candida albicans, microorganisms which preferentially colonize skin and mucosa, for being susceptible to CXCL14/BRAK. The antimicrobial activity of CXCL14 was evaluated by the radial diffusion assay and was compared to that of the chemokines CCL20, CCL27, CXCL8 and to human beta-defensin-2, a prototype antimicrobial peptide in skin. We found that CXCL14/BRAK exhibits strong activity against Escherichia coli, Staphylococcus aureus, coagulase-negative Staphylococci, Propionibacteria and Candida albicans. CXCL14/BRAK was more potent against these organisms than the other chemokines and also more potent than human beta-defensin-2. A relevant antimicrobial effect of CXCL14/BRAK against Escherichia coli was observed at a concentration as low as 50nM. Moreover, culture supernatants of CXCL14/BRAK-transfected cell lines exhibit significantly stronger antimicrobial activity than supernatants of respective parental cell lines. The antimicrobial activity is highly specific for CXCL14/BRAK and is reduced to a large extent by adding an anti-CXCL14 antibody.
Altogether, we describe an important new function of CXCL14/BRAK by showing that this chemokine exhibits strong antimicrobial activity and therefore may be involved as a local host defence molecule protecting healthy skin and possibly other epithelia from infection by various microbes.

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Content differences in health status measures and withdrawal scales used for smoking cessation

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Background: Quality of life assessments in smoking cessation programs have increasingly gained importance, especially when evaluating treatment interventions. In this context the International Classification of Functioning, Disability and Health (ICF) serves as an appropriate tool for comparative purposes. The ICF helps to understand the relationship between the different health-related quality of life (HRQL) instruments when systematically assessing their contents. The objective of this study was therefore to compare the content of HRQL instruments and withdrawal scales applied in the field of smoking cessation using the ICF as a reference.
Methods: Twelve smoking-specific instruments, mentioned in widely accepted guidelines, were linked to the ICF by various health professionals using standardized linking rules. The degree of agreement was assessed by kappa statistics.
Results: Instruments varied strongly in the number of concepts contained and the ICF categories used to map these concepts. In total, 281 concepts were identified and linked to 20 ICF categories. However, of these 281 original concepts 249 concepts were linked to 8 categories of the chapter 'mental functions'. Solely the category 'Energy and drive functions', covering 'Craving'was covered by all instruments. Only the further two categories 'Sleep functions', and 'Attention functions' were covered by more than half the instruments. While we found most similarities within the ICF chapter mental functions, there are striking differences in what is considered of further relevance within the component body functions. The remaining contents were very heterogeneously covered and weighted: 9 categories were unique to specific instruments. Most interestingly, environmental factors are only covered by two instruments.
Conclusion: This study generated an inventory, which may serve to compare the HRQOL instruments used in smoking cessation programs. The results of this study may help to guide researchers and clinicians to choose the most appropriate instrument for specific purposes in clinical and research settings.

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Production and characterisation of rationally optimised, anti-melanoma T-cell receptors: a step closer towards immunotherapeutic treatment for patients

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Background: In general, cytotoxic T cells are poor at mounting effective anti-tumor responses against tumor associated antigens (TAAs). This may, in part, be due to the deletion of high affinity anti-TAA T cell receptors (TCRs) during negative selection in the thymus. Here we describe the production and preliminary characterization of a rationally optimized anti-NY-ESO-1/A2 TCR. This TCR is considered clinically relevant because it was cloned from an immunodominant T cell clone derived from a long-surviving melanoma patient. Ultimately it is hoped that such optimized TCRs can be used in the immunotherapeutic treatment of patients.
Methods: The TCR-pMHC complex was modeled in silico and point mutants were identified which were predicted to increase TCR binding to pMHC. In order to test these TCR mutants for improved binding they were produced solubly along with the parental TCR. The alpha and beta chains were cloned into pHYK8 vectors and co-transfected into HEK-293 cells. To facilitate heterodimer formation, an acidic-basic zipper was incorporated at the ends of the truncated chains. A HIS tag was included for purification via nickel agarose or metal chelate membrane adsorbers. Transfected cells were grown in suspension in serum-free medium for 5–7 days and yields of purified TCR ranged from 1.5–10 mg/L. A direct ELISA, in which soluble TCR was titered against pMHC, was used to screen for optimized mutants that would further undergo affinity and kinetic measurements using the BIAcore instrument.
Results: From the first set of mutations tested we identified two single point mutations in the CDR2 beta region that resulted in improved binding, as compared to the parental TCR, to pMHC. The two mutations were combined and the resultant TCR showed further improved binding. We are currently conducting our second round of point mutations. Preliminary BIAcore work revealed incomplete dissociation of the TCRs from the coated chip, presumably due to non-specific binding of the zipper. Strategies to overcome this obstacle are being investigated.
Conclusion: The mammalian expression system that we have developed for the production of properly folded, soluble TCR, has enabled a fast and efficient means of evaluating our in silico modeling work on the rational optimization of TCR-pMHC interactions. Our next step is to investigate the affinity of the parental and optimized TCR mutants and evaluate the contributions of on-rates and off-rates to the improved binding.

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Toll-like receptor induced expression of hypoxia-inducible factor 1a in human dendritic cells

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Introduction: Non-hypoxic stimuli can induce the expression of hypoxia-inducible factor 1a (HIF-1a) under normal oxygen conditions. Only recently it has been demonstrated that lipopolysaccharide (LPS) induces the expression of HIF-1a in macrophages, and that the induction of hypoxic genes in macrophages is toll-like receptor 4 (TLR-4) dependent. We hypothesized that HIF-1a expression is induced in dendritic cells (DC) in a TLR-dependent manner, plays a crucial role in linking the innate with the adaptive immune system and may also influence mitochondrial respiration.

Material and methods: Human monocyte-derived immature DC were stimulated with different TLR-2 and 4 ligands (hyaluronic acid [HA], LPS or lipoteichoic acid [LTA]) under normoxia. Furthermore, we incubated DC under hypoxic conditions (1.5% oxygen) with or without stimulation with LPS. HIF-1a expression was examined by western blot at 2h, 4h, 6h, 8h, 12h and 24h after TLR stimulation. DC were analyzed for the expression of the co-stimulatory molecules and maturation markers CD80 and CD86 by flow cytometry (FACScan, B&D). Mitochondrial respiration of digitinon-permeabilized cells was determined using a High Resolution Oxygraph (Oroboros Instruments, Innsbruck, Austria) and DatLab 4.2 software for data acquisition and analysis.
Results: All tested TLR agonists stimulated the expression of HIF-1a in a time-dependent manner. Interestingly, TLR induced HIF-1a expression levels in normoxia were even higher than in hypoxia. HA, LPS and LTA led to DC maturation, as shown by the up-regulation of CD80 and CD86 expression. LPS also increased complex II-dependent mitochondrial respiration of DC.
Conclusions and Outlook: The current data demonstrate that HIF-1a expression in DC is induced under normoxic conditions via TLR-2 and -4 agonists in a time-dependent manner. Furthermore, LPS treatment leads to an increased complex II-dependent mitochondrial respiration. In further investigations we will examine if a HIF-1a knock-down in human DCs by siRNA leads to an impaired immunostimulatory capacity measured by the ability to activate T cells and if TLR ligation leads to a HIF-1a-dependent release of cytokines and growth factors and modification of mitochondrial respiration.

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Crosstalks between neutrophils and dendritic cells during leishmania major infection

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Neutrophils are key components of the inflammatory response and contribute to the development of pathogen-specific immune response. Neutrophils are recruited within hours of an infection with Leishmania major (L. major). C57BL/6 mice are resistant to infection with L. major developing a protective Th1 response, while BALB/c mice, developing a Th2 response, are susceptible to infection and do not control parasite replication nor healing of lesions. Dendritic cells play a crucial role in initiating and regulating the T cell immune response. Uptake of L. major by dendritic cells results in their activation and IL-12 secretion, a cytokine which is critically involved in the Th1 induced killing of the parasite. In this report, we investigated in vitro and in vivo, the crosstalks between neutrophils and dendritic cells following L. major infection in C57BL/6 and BALB/c mice. The secretion of dendritic cells attracting chemokines by neutrophils was first investigated. C57BL/6 neutrophils transcribed and secreted significantly higher levels of CCL3, CCL4 and CCL5 chemokines in response to L. major compared to BALB/c neutrophils. In vitro migration assay showed that the higher secretion of dendritic cells attracting chemokines by C57BL/6 neutrophils correlated with a higher migration of dendritic cells towards C57BL/6 neutrophils supernatant as compared to that of BALB/c neutrophils. Following s.c. injection of L. major, 24 hours after infection, a significantly higher amount of dendritic cells were found to be transiently recruited to the site of injection in resistant C57BL/6 mice compared to susceptible BALB/c mice. Depletion of neutrophils prior to infection led to inhibition of the migration of dendritic cells to the site of infection, suggesting that neutrophils are essential for early cellular recruitment. Altogether, these results indicate that the differential release of dendritic cells attracting chemokines by neutrophils following infection with L. major induces the migration of dendritic cells to the site of infection and thus may influence L. major-specific immune response.

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Leishmania major-specific B-cells are necessary for Th2 cell development and susceptibility to L. major LV39 in BALB/c mice

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B lymphocytes are considered to play a minimal role in host defence against L. major. In this study, the contribution of B cells to susceptibility to infection with different strains of L. major was investigated in BALB/c mice lacking mature B cells due to the disruption of the IgM transmembrane domain (uMT). Whereas BALB/c uMT remained susceptible to infection with L. major IR173 and IR75, they were partially resistant to infection with another strain of L. major, L. major LV39. Adoptive transfer of naïve B cells into BALB/c mMT mice prior to infection restored susceptibility to infection with L. major LV39, demonstrating a role for B cells in susceptibility to infection with this parasite. The two main functions of B cells are Ig production and/or Ag presentation to T cells. First, by transfer of immune serum in BALB/c uMT mice, we demonstrate that specific Ig did not contribute to disease progression. Thus, the role of APC function of the transferred B cell on the expression of a susceptible phenotype in resistant BALB/c uMT mice following adoptive transfer of B cells was evaluated. Adoptive transfer of B cells that express an IgM/IgD specific for HEL, an irrelevant antigen, did not restore disease progression in BALB/c uMT mice infected with L. major LV39. This was likely due to the inability of HEL Tg B cells to internalize and present Leishmania antigens to specific T cells. These data suggest that direct antigen presentation by specific B cells and not Ig effector functions is involved in susceptibility of BALB/c mice to infection with L. major LV39.

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In vitro drug-induced cytokine release from peripheral blood mononuclear cells of patients with delayed-type drug hypersensitivity

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The most prevalent hypersensitivity reactions to drugs are T-cell mediated. The only established in vitro test for detecting T cell sensitization to a drug is the lymphocyte transformation test (LTT), which measures the proliferative response of T cells to the drug. LTT involves the use of radioactivity and is time-consuming; therefore its practicability is limited. Our goal was to find an alternative in vitro method to detect drug-sensitized T cells. We analyzed in vitro the secretion pattern of 17 cytokines and chemokines from peripheral blood mononuclear cells (PBMCs) of patients with well-documented drug allergies (MPE and DRESS), in order to identify those cytokines which are predominantly secreted upon exposure to the relevant drug. PBMCs of 5 amoxicillin-allergic patients, 5 sulfamethoxazole-allergic patients and 5 healthy controls were incubated for 3 days with the drug antigen. Cytokine concentrations were measured in the supernatants using commercially available 17-plex, multiplex, bead-based immunoassay kits. Among the 17 cytokines/chemokines analyzed, IL-5, IL-13 and IFN-gamma secretion in response to the drug antigens was increased in patients' PBMCs compared to healthy controls. No difference in cytokine secretion patterns between sulfamethoxazole- and amoxicillin-reactive PBMCs could be observed. The secretion of other cytokines, such as the Th1 cytokines IL-2 and IL-12, and the Th2 cytokines IL-4 and IL-10, showed a high variability among patients. We conclude that from the 17 cytokines/chemokines analyzed, the measurement of IL-5, IL-13 and IFN-gamma might be a useful tool for in vitro detection of T cell sensitization to drugs. Secretion of these cytokines is independent of the drug antigen and the phenotype of the drug reaction.

P198

CD4+ T-cell help improves CD8+ T-cell memory by retained CD27 expression

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CD4+ T cell help during the priming of CD8+ T lymphocytes imprints the capacity for optimal secondary expansion upon re-encounter with antigen. Helped memory CD8+ T rapidly expand in response to a secondary antigen exposure, even in the absence of T cell help and, are most efficient in protection against a re-infection. In contrast, helpless memory CTL can mediate effector function, but secondary expansion is reduced. How CD4+ T cells instruct CD8+ memory T cells during priming to undergo efficient secondary expansion has not been resolved in detail. Here we show that memory CTL after infection with lymphocytic choriomeningitis virus (LCMV) are CD27high whereas memory CTL primed in the absence of CD4+ T cell have a reduced expression of CD27. Helpless memory CTL

produced low amounts of IL-2 and did not efficiently expand after restimulation with peptide in vitro. Blocking experiments with monoclonal antibodies and the use of CD27-/- memory CTL revealed that CD27 ligation during re-stimulation increased autocrine IL-2 production and secondary expansion. Therefore, regulating CD27 expression on memory CTL is a novel mechanism how CD4+ T cells control CTL memory.

P199

Innate immune responses of macrophages and dendritic cells to poxvirus

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Background: Attenuated NYVAC and MVA poxvirus strains are currently used for vaccine development against a broad spectrum of diseases. Whilst these vectors have been shown to be immunogenic and safe in humans, the innate immune responses they elicit remain largely unknown.
Aim: To assess the response of human THP-1 cells and mouse dendritic cells to poxviruses infection and to elucidate the role of the Toll-like-receptor (TLR) pathways in that response.
Results: MVA-stimulated THP-1 monocytes secreted large amounts of chemokines (MIP-1, MCP-1, IP-10, RANTES), but low levels of pro-inflammatory cytokines (TNF, IL-6, IL-1beta). In contrast, NYVAC induced weak production of both cytokines and chemokines in THP-1 cells. Using wild-type and TLR2, TLR4, MyD88 and TRIF deficient mouse dendritic cells, we observed that TLR2-MyD88 was essential for production of chemokines, but dispensable for IFNbeta production, after stimulation with MVA.
Conclusion: MVA triggers innate immune responses of macrophages and dendritic cells via TLR-dependent and TLR-independent pathways. Work is in progress to identify the TLR-independent pathway(s) activated by MVA.

P200

A critical lineage-nonspecific role for pT alpha in mediating allelic and isotypic exclusion in TCR beta transgenic mice

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Although it is well established that early expression of TCRbeta transgenes in the thymus leads to efficient inhibition of both endogenous TCRbeta and TCRgamma rearrangement (also known as allelic and isotypic exclusion, respectively) the role of pTalpha in these processes remains controversial. In order to re-evaluate this issue in a systematic manner we have utilized three independent TCRbeta transgenic mice that differ widely in transgene expression levels, and a sensitive intracellular staining assay that detects endogenous TCRVbeta expression in individual immature thymocytes. In the absence of pTalpha both allelic and isotypic exclusion were reversed in all three TCRbeta transgenic strains, clearly demonstrating a general requirement for pre-TCR signaling in the inhibition of endogenous TCRbeta and TCRgamma rearrangement. Both allelic and isotypic exclusion were pTalpha dose-dependent when transgenic TCRbeta levels were sub-physiological. Moreover, pTalpha dependent allelic and isotypic exclusion occurred in both alpha/beta and gamma/delta T cell lineages, indicating that pre-TCR signaling can potentially be functional in gamma/delta precursors. Finally, using real-time PCR and western blot techniques we found that allelic and isotypic exclusion in DN3 thymocytes can occur independently of regulation of RAG expression.
In conclusion, our data reveal a critical but lineage-nonspecific role for pTalpha in mediating both allelic and isotypic exclusion in TCRbeta transgenic mice.

P201

Haematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair

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Adult bone marrow hematopoietic stem cells (HSCs) are crucial to maintain lifelong production of all blood cells and have been suggested to divide regularly but infrequently. Using multi-parameter flow cytometry and label retaining assays (BrdU and HistoneH2B-GFP), we identify a rare population of dormant HSCs (d-HSCs) harboring the vast majority of multi-lineage long-term self-renewal activity as shown by serial transplantation. Mathematical modeling suggests that d-HSCs comprise about 15% of the Lin-Sca1+cKit+CD150+CD48-CD34- population with a doubling time of approximately 140 days. d-HSCs form a silent reservoir of highly potent HSCs during homeostasis, but are efficiently activated to self-renew upon bone marrow injury induced by 5-fluorouracil or BrdU. After homeostasis is re-established proliferating HSCs return to dormancy, suggesting that HSCs reversibly switch from a dormant to a self-renewing phase under conditions of hematopoietic stress.

P202

Relationship between cytotoxicity and proliferative capacity in virus-specific CD8 T-cells

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Background: Virus-specific CD8 T cells display several functions including notably cytotoxicity and proliferation capacity. The relationship between these functions remains unclear in humans. Of note, T-cell responses against different viruses can be used to evaluate the influence of the antigen load on the response.
Methods: A variety of virus-specific CD8 T-cell responses including CMV (n = 14), EBV (n = 7), Flu (n = 5) and HIV-1 from both progressors and nonprogressors (n = 11) were identified using tetramer complexes and analyzed for proliferation capacity and expression of perforin (used as a marker of cytotoxic cells) and CD127 (i.e. IL-7Ra, used as a marker of cells endowed with proliferation capacity). CCR7 and CD45RA were used to assess T-cell differentiation.
Results: Combined expression of perforin and CD127 revealed the existence of three populations of CD8 T-cells: CD127+perforin-, CD127-perforin- and CD127-perforin+ which were CD45RA-CCR7+, CD45RA-CCR7- and CD45RA+CCR7- CD8 T-cells, respectively. Of note, virus-specific proliferating CD8 T-cells were contained within the CD127+perforin- cells population. Furthermore, CD127+perforin- cells represented the large majority (90%) of Flu-specific CD8 T-cells; CD127-perforin- cells were the majority (64%) of EBV-specific CD8 T-cells; and CD127-perforin+ cells were dominant for CMV-specific CD8 T-cells (43%). These differences were significant (all $P < 0.05$). HIV-specific CD8 T-cells from progressors were mostly CD127-perforin- cells while nonprogressors had significantly more CD127+ CD8 T-cells (21 vs. 7%, $P < 0.01$). In contrast, progressors had more perforin+ cells than nonprogressors (10 vs. 4%). Of interest, 7 days after in vitro antigen-specific stimulation, the CD127-perforin+ T-cell subset significantly increased for all virus-specific CD8 T-cell responses: Flu: from 2 to 42%; EBV: from 3 to 45%; CMV: from 43 to 60%; HIV: from 10 to 25%; (all $P < 0.05$). Interestingly, the up-regulation of perforin following specific in vitro stimulation on Flu-specific CD8 T cells correlated with the acquisition of direct cytotoxic activity.
Conclusions: Cytotoxic and proliferative CD8 T cells are distinct T-cell subsets that are at different stages of differentiation. The balance between proliferative/precursor and cytotoxic/effector subsets of virus-specific CD8 T-cells is very heterogeneous among the different models of virus infection and appears to be influenced by the antigen load/exposure.

P203

Relationship between HLA genotype and the functional profile of Ag-specific CD8 T-cells

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Background: Polyfunctional (IFN-g + IL-2 secretion and proliferation) and not monofunctional (IFN-g secretion) CD8 T-cell responses are associated with protective antiviral immunity and nonprogressive HIV disease. On the other hand, HLA-B influences the outcome of HIV disease. In this study, we have investigated the relationship between the HLA genotype and the functional profile of CD8 T-cells.
Methods: We performed a comprehensive characterization of HLA genotype (4-digit) and of the functional profile of virus-specific CD8 T-cell responses against HIV-1, CMV, EBV and Influenza (Flu) in 177 subjects comprising 69 HIV negative, 99 subjects with chronic progressive HIV-1 infection, 9 nonprogressors and 34 subjects with acute HIV infection.
Results: Gag (n = 89) responses, obtained from 44 experimentally confirmed peptide-HLA associations, were identified and characterized in HIV-1-infected progressors. HLA-B-restricted epitopes were associated more frequently with polyfunctional CD8 T-cell responses than HLA-A-restricted epitopes (P = 0.008). In addition, in a panel of 13 responses derived from gag epitopes restricted either by HLA-A or HLA-B alleles, polyfunctional CD8 T-cell responses were associated with HLA-B-restriction (P = 0.002). Of note, monofunctional HLA-A-restricted and polyfunctional HLA-B-restricted responses were simultaneously observed within the same subjects. Furthermore, HIV-1- (in LTNP), EBV-, CMV- and Flu-derived HLA-B-restricted responses were significantly more polyfunctional than HLA-A-restricted responses (P = 0.03, 0.02, 0.02 and 0.005, respectively). Of interest, HLA-B-restricted responses were associated with lower avidity, lower differentiation state and lower PD-1 level of expression as compared to HLA-A-restricted responses (P = 0.004, 0.004, 0.008, respectively). Of note, a significant correlation was observed between PD-1 expression and the proportion of HIV-1-specific IL-2 secreting CD8 T cells (P <0.001). Finally, HIV-infected patients treated at the time of the acute infection had more polyfunctional CD8 T-cell responses with lower avidity as compared to patients treated during chronic infection.
Conclusion: These results provide new insights into the associations between HLA restriction, TCR avidity, PD-1 expression and the functional profile of virus-specific CD8 T cell responses. Furthermore, they provide the rationale for the protective role of HLA-B in HIV-1-infection.

P204

Recombinant Vaccinia virus expressing HSV-ICP47: hiding vector epitope for an increased efficiency as cancer vaccine

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Immunodominant vector-specific CTL responses could limit their effectiveness especially in cancer immunotherapeutic strategies implying multiple rounds of vaccine stimulations. We aim at decreasing CTL responses against Vaccinia Virus by diminishing the viral epitope MHC class-I restricted presentation from infected cells. ICP47 protein (encoded by US12 gene from HSV-I) is inhibiting TAP-dependant transport to the endoplasmic reticulum (ER) of processed peptide thus preventing their class-I presentation. We hypothesized that co-expression of HSV-ICP47 in a recombinant vaccinia virus (rVV) would prevent presentation of viral proteins without affecting the presentation of recombinant TAP-independent tumor associated antigen (TAA) epitopes. MHC class-II presentation of viral entities, which play a major role as "helper signal" during the generation of CD8+ response, should not be affected.
Methods: Herpesvirus US12 gene was inserted into wild type genome as well as into a rVV expressing ER-Mart27-35. Replication of the virus is prevented by psoralen-UV treatment. Effect on MHC-class I of infected cells was characterized by antibody staining and FACS analysis.
Human T-lymphocytes were stimulated in vitro with autologous CD14+ cells infected with US12-rVV, M-US12- rVV or control virus. Proliferation of specific CD8+ and CD4+ for viral proteins and the recombinant epitope were monitored by MHC-multimer and IFNg intracellular staining.
Results: Already 16-24h after infection, cells transfected with US12-rVV demonstrated a clear MHC class-I downregulation. In HLA-A0201 positive cell lines, downregulation of the HLA-A2 complex observed with US12-rVV was partially compensated by the co-expression of ER-Mart27-35 peptide encoded in M-US12-rVV. No effect of US12-rVV was seen for other surface molecules such as CD44, CD80 and MHC class-II, confirming the specificity of the blockade. Finally, preliminary tests seem to indicate that CD8+ responses against viral epitopes (processed from vaccinia vector) are diminished when primed with US12-rVV.
Conclusion: Recombinant vaccinia virus expressing the HSV-US12 gene confirmed a diminished class-I presentation of native viral proteins from infected cells. Whereas the immunogenicity of recombinant ER-targeted class-I TAA epitope, as well as viral helper class-II entities, should be conserved. Such reagent could become of high relevance especially in multiple-boost vaccine protocol required in cancer immunotherapy.

P205

Effective T-cell immune responses in the absence of the serine/threonine kinase RIP2

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The serine/threonine kinase RIP2 has been reported to be essential for Nod1 and Nod2 mediated cell activation, and has been suggested to play a role in the signaling cascade downstream of the T cell receptor. We sought to ascertain the exact role of RIP2 in T helper cell differentiation and CD8+ T cell effector function in vivo and in vitro. In contrast to previous reports, we found that RIP2 deficient T cells did not exhibit impaired proliferation upon TCR engagement in vitro, and differentiation to cytokine producing Th1 or Th2 cells was normal in the absence of RIP2. These results were confirmed in vivo, as wild type and RIP2 deficient virus-specific CD8+ T cells expanded comparably in mice after LCMV infection. Wild type and RIP2 deficient CD4+ and CD8+ T cells from infected mice also showed similar proliferation and cytokine production when restimulated with full or partial agonist peptides ex vivo. Furthermore, no significant difference in adaptive T cell responses could be observed between wild type and RIP2 deficient mice after Listeria monocytogenes infection. Thus contrary to early reports, our data shows that RIP2 is not an essential component of the TCR signaling machinery.

P206

Unravelling the mystery in behind amoxillin rash in EBV infection

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Background: Teenagers and young adults suffering from acute infectious mononucleosis (IM) develop maculopapular exanthema following amoxicillin or ampicillin ingestion in nearly 100% of the cases. In several reports, lymphocytes transformation test and/or skin tests revealed that affected individuals remained sensitized to the drug after the acute infection. In this study we investigate whether EBV specific T cells, which are present in high amount during the acute infection, may also react with amoxicillin.
Methods: Autologous EBV transformed B cell lines (B-LCL) were used to expand EBV specific T cells in in-vitro cultures of PBMC of healthy donors. Expansion was monitored by CFSE staining; EBV specificity and amoxicillin reactivity were explored by intracellular staining for IFNg and IL-2 at day 7, 14, 28 and 56 of the EBV specific expansion.
Results: In 2 donors with a history of IM (but no amoxicillin rash), 2.2 to 6.3% of CD8+ T cells secreted IFN$f\times$ in response to amoxicillin at day 14 and 28 of the EBV specific expansion. No amoxicillin reacting T cells could be identified in PBMC analysed ex-vivo, confirming the necessary EBV expansion for amoxicillin reactivity. In order to characterize more precisely the possible hapten and peptide cross-reactivity of such cells, T cells of these 2 donors were cloned. Among the numerous EBV specific T cell clones generated, seven were also reacting to amoxicillin. Identification of the involved viral peptides as well as the restriction of such cross-reactivity are currently under investigation.
Conclusion: This finding sheds new light on the amoxicillin rash in acute EBV infection and suggests that this phenomenon might be due to the stimulation by EBV of T cells cross reactive with viral peptides and amoxicillin.

P207

Type I interferon-mediated protection of macrophages and dendritic cells secures control of murine coronavirus infection

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The swift production of type I interferons (IFNs) is one of the fundamental aspects of innate immune responses against viruses. We have shown previously that plasmacytoid dendritic cell (pDC)-derived type I IFNs are of prime importance for the initial control of mouse hepatitis virus (MHV) infection (Cervantes-Barragan et al. 2007, Blood 109:1131). In the present study, we have determined the major target cell populations of this first wave of type I IFNs. Generation of bone marrow chimeric mice expressing the type I interferon receptor (IFNAR) either on hematopoietic or non-bone marrow-derived cells revealed that the early control of MHV depended mainly on IFNAR expression on hematopoietic cells. To establish which cell population responds most efficiently to type I IFNs, mice conditionally deficient for the IFNAR on different leukocyte subsets were infected with MHV. This genetic analysis revealed that IFNAR expression on macrophages and CD11c+ dendritic cells (DCs) was most important for the early containment of MHV within secondary lymphoid organs and to prevent lethal liver disease. Taken together, this study identifies type I IFN-mediated cross-talk between pDCs on one side, and macrophages and conventional DCs on the other, as an essential cellular pathway for the control of cytopathic virus infection.

P208

In vitro induction of mucosa-type dendritic cells by all-trans retinoic acid

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Intestinal dendritic cells (DC) display a unique ability to promote mucosal immune responses by inducing the secretion of IgA and the expression of gut-homing receptors on lymphocytes. This particular capacity is associated with the selective expression of Vitamin A converting dehydrogenases and the production of the active metabolite all-trans retinoic acid (RA). However, intestinal DC may not only be producers but could also represent targets of RA derived from enterocytes and/or RA provided in an autocrine manner. We therefore investigated whether exposure of peripheral DC to RA endows them with attributes of mucosal DC. Porcine monocyte-derived dendritic cells (MoDC) pre-treated with RA produced increased TGFbeta and IL-6 compared to untreated MoDC. Intriguingly, production of IL-6, but not expression of IL-12 mRNA, was augmented several-fold when MoDC were co-treated with TLR ligands and RA. MoDC pre-treated with RA induced upregulated expression of gut-homing receptors and increased IgG and IgA secretion in co-cultured lymphocytes. While the induction of upregulated alpha4beta7 integrin expression was dependent on TGFbeta and residual RA, presence of a retinoic acid receptor (RAR) antagonist did not impair the increased immunoglobulin secretion, indicating a role for yet unidentified factors derived from RA-treated MoDC. We further showed that exposure of MoDC to physiological concentrations of RA induced the expression of retinaldehyde dehydrogenase 1a1 (RALDH1) mRNA. Importantly, culture of MoDC in the presence of supernatants derived from intestinal in vitro organ cultures (IVOC) also induced RALDH1 mRNA expression. RALDH1 mRNA induction by IVOC-supernatants was abolished in the presence of a RAR antagonist. Collectively, these results suggest that epithelial-derived RA may play a role in imprinting DC with mucosal attributes including the expression of RALDHs.

P209

SCART scavenger receptors identify a novel subset of adult gamma-delta T-cells

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T lymphocytes are composed of two main lineages: the alpha-beta (AB) and the gamma-delta (GD) cells. Both of them exist in all vertebrates, however their ratio changes dramatically depending on the organism and tissue studied. Only around 1–5% of total T cells in peripheral lymphoid organs are GD T cells, however in some tissues GD T cells can represent 20–50% of lymphoid cells and carry out protective or immunoregulatory functions.
While there has been a great progress in the characterization of AB T cell differentiation, selection and function, GD T cells remain poorly understood. One of the main reasons for this is the lack of GD T cell specific surface markers other than TCR chains themselves. Here we describe two novel surface receptors, SCART1 and SCART2, which identify a new, IL-17 producing, subset of GD T cells. SCARTs are related to CD5, CD6 and CD163 scavenger receptors but, unlike them, they mark developing and mature GD T cells. Characterization of SCART2 positive immature and peripheral GD T cells suggests that they undergo lineage specification in the thymus and belong to a new subset with distinct functional and homing capabilities.

P210

Carrier induced epitopic suppression is a dynamic phenomenon caused by carrier specific antibodies

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Pre-existing immunity against vaccine carrier proteins have been reported to inhibit the immune response against antigens conjugated to the same carrier by a process termed carrier induced epitopic suppression (CIES). Hence understanding the phenomenon of CIES is of major importance for the development of conjugate vaccines. Virus-like particles (VLP) are a novel class of potent immunological carriers which have been successfully used to enhance the antibody response to virtually any conjugated antigen. In the present study we investigated the impact of a pre-existing carrier-specific immune response on the development of antibody responses against a conjugated model peptide after primary, secondary and tertiary immunization. Although carrier specific immune responses led to reduced peptide specific antibody titers, we showed that CIES could be overcome by high coupling densities, repeated injections and/or higher doses of conjugate vaccine.
Furthermore we dissected carrier specific immunity by adoptively transferring each component of immunity (antibodies, B-cells, Thelper cells) separately into naïve mice and found that the observed CIES was mainly mediated by carrier specific antibodies.

P211

Lentivector immunisation induces tumour antigen-specific T- and B-cell responses in vivo

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Expression of the cancer/germline antigen NY-ESO-1 by tumors elicits spontaneous humoral and cellular immune responses in some cancer patients. It is desirable to develop vaccines capable of stimulating such comprehensive immune responses. We produced recombinant lentivectors directing the intracellular synthesis of NY-ESO-1. Single injection of this lentivector into HLA-A2 transgenic mice elicited long-lasting B- and T-cell responses against NY-ESO-1. CD8+ T cells against the HLA-A2-restricted peptide NY-ESO-1(157-165) were readily detectable ex vivo and showed restricted TCR β usage. Moreover, lentivecor elicited a far stronger anti-NY-ESO-1(157-165) CD8+ T-cell response than peptide or protein-based vaccines. Anti-NY-ESO-1 antibodies were rapidly induced after immunization and preceded the CD8+ T-cell response, suggesting that the antibody response might be an important component of the anti-NY-ESO-1 CD8+ T-cell response in vivo. CD4+ T-cells also played a crucial role as their depletion completely abrogated both B-cell and CD8+ T-cell responses against NY-ESO-1. The induced CD4+ T-cell response was primarily directed against a single NY-ESO-1 epitope (spanning amino acids 81-100). Described lentivector-based immunization protocol induced complex immune responses comparable to those described in humans with NY-ESO-1-positive tumors. It also revealed a possible mechanism by which anti-NY-ESO-1 CD8+ T cell responses may be elicited in vivo.

P212

Bcl10 phosphorylation plays a key role in T-cell receptor-induced actin polymerisation

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The protein B-cell lymphoma/leukemia-10 (Bcl10) was originally identified from a chromosomal translocation that is associated with formation of B-cell lymphomas of the mucosa-associated lymphoid tissue (MALT-lymphomas). It is thought that this chromosomal translocation brings the BCL10 gene into proximity of the immunoglobulin enhancer element and results in over-expression of Bcl10, but it is unknown how this contributes to cellular transformation.
Bcl10 also plays a key role in antigen receptor-mediated lymphocyte activation, which depends on its association with the proteins Carma1 and Malt1. Antigen receptor stimulation leads to the PKC-dependent formation of a Carma1-Bcl10-Malt1 complex that is critical for IKK-mediated NF-kB activation and for activation of the JNK pathway. Consequently, lymphocytes from mice with compromised expression or function of Carma1, Bcl10 or Malt1 are immuno-deficient because of impaired antigen receptor-induced NF-kB and JNK activation that leads to impaired cytokine production and proliferation. While these findings support a key role for Bcl10 and its binding partners in the regulation of the NF-kB and JNK transcriptional pathways, it is likely that Bcl10 also has other, transcription-independent functions and/or functions that are independent of its interaction with Carma1 and Malt1.
Upon lymphocyte activation, Bcl10 undergoes multiple post-translational modifications, but the physiological relevance of these modifications for the molecular function of Bcl10 is not precisely understood. We have started to characterize these post-translational modifications and identified Ser 138 as a key residue necessary for Bcl10 phosphorylation upon activation of human primary and Jurkat T cells by PMA/ionomycin- or anti-CD3 treatment. Interestingly, a phosphorylation-deficient Ser 138/Ala mutant specifically inhibited T-cell receptor (TCR)-induced actin polymerization yet did not affect NF-kB or JNK activation. Moreover, silencing of Bcl10- but not of Carma1- expression induced a clear defect in TCR-induced F-actin formation, cell spreading and conjugate formation in Jurkat T cells. Remarkably, Bcl10 silencing also impaired Fc-gamma receptor-induced actin polymerization and phagocytosis in human monocytes. Together, these results identify a key role of Bcl10 in the regulation of F-actin-dependent cellular functions of leukocytes that are independent of the Bcl10 interaction partners Carma1 and Malt1.

P213

The dual role of CD27 signaling in tumour surveillance and promotion

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Introduction: CD27 is a member of tumor necrosis factor receptor (TNFR) family and expressed on T cells, activated B cells and some NK cells. Its ligand CD70 is tightly controlled and expressed on activated T and B cells and dendritic cells (DC). CD27 activation results in NFkB activation, enhances TCR-mediated expansion and survival and increases effector function. In contrast, NFkB-mediated expression of inflammatory cytokines supports proliferation and survival of tumor cells and improves angiogenesis. Why in some situations CD27 ligation improves T cell responses and tumor control whereas in other situations it has detrimental consequences for the host is unkown.
Method: We analyzed anti-tumoral immune responses and tumor control in murine models. We injected carcinogenic 3-methyl-cholantrene (MCA) or transplanted tumor fragments subcutaneously in wildtype (WT) mice and CD27-/- mice and observed tumor development and growth. The analyzed tumor cell lines included murine fibrosarcoma MC57, colon adenocarcinoma MC38 and melanoma B16F10. Depletion of defined lymphocyte populations or adoptive transfer of CD27+ lymphocyte populations in CD27-/- mice were used to define the responsible lymphocyte population that promotes tumor growth after CD27 ligation.
Results: Comparable to earlier studies we found that the tumor-specific CTL response after injection of tumor cells in single cell suspension is reduced in CD27-/- mice. In contrast and surprisingly the tumor growth of transferred tumor fragments was reduced in CD27-/- mice when compared with WT mice. Similarly, spontaneous tumor development and growth after MCA treatment was reduced. CD70 expression was hardly detectable in spleen and tumor-draining lymph nodes but was highly expressed on tumor infiltrating DCs, CD4+ and CD8+ T cells. Depletion of CD4+ T cells resulted in rejection of MC57 tumor in WT and CD27-/- mice. Adoptive transfer of CD27+ CD4+ T cells in CD27-/- mice enhanced tumor gowth.
Conclusion: Our results suggest that persistent CD27 signalling on tumor-infiltrating CD4+ T cells increases the secretion of proinflammatory cytokines such as TNFa and IFNg, which support tumor development and progression.

P214

The inhibitor of histone deacetylases (HDI) trichostatin A (TSA) down-regulates macrophage migration inhibitory factor (MIF) gene expression through a local deacetylation of the MIF promoter

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Background: HDIs are currently being tested as anti-cancer agents in clinical trials. Yet, the molecular basis of the anti-tumoral effects of HDIs is not fully understood. Recent studies have highlighted that HDIs may also inhibit inflammatory responses. MIF, a pro-inflammatory cytokine, has recently been implicated in cell proliferation, angiogenesis and oncogenesis and may therefore represent a potential target of HDI action.
Objective: To study whether HDIs modify the expression of MIF in human cells and, if so, to identify the molecular mechanism(s) underlying this inhibition.
Methods: Human whole blood, HeLa cervix epithelial cells, HaCat keratinocytes and U-937, KG1a and HL-60 leukemic cell lines were exposed to TSA. In selected experiments, HeLa cells were infected (MOI100) with a control adenovirus or a MIF-encoding adenovirus (MIF-Ad). MIF gene transcription, mRNA and protein expression were assessed by nuclear run on, Northern blotting and Western blotting. Transcription factor binding activity and histone acetylation were assessed by chromatin immunoprecipitation (ChIP). Chromatin accessibility was quantified by CHART (chromatin accessibility by real-time).
Results: TSA strongly inhibited MIF mRNA expresion in all cell lines, and reduced MIF protein expression by whole blood and HeLa cells. TSA reduced 2-fold MIF gene transcription. Even though global histone acetylation was robustly increased by TSA, TSA deacetylated the histone H3 associated with the MIF promoter. This effect did not affect chromatin accessibility to the MIF gene, but was coupled with a decreased recruitment of the basal transcriptional machinery (Sp1, CREB and RNA pol II) to the bona fide MIF promoter. Finally, TSA did not reduce adenoviral-derived MIF expression, confirming that TSA exerted its inhibitory effect on MIF expression at the level of chromatin.
Conclusions: TSA downregulates MIF expression by a molecular mechanism involving a local deacetylation of MIF-associated histone H3 and a reduced transcription of the MIF gene. Considering that MIF is over-expressed in human neoplasia and required for tumor associated angiogenesis, our findings suggest that the anti-tumoral effects of HDI may be mediated by a down-regulation of MIF expression. Moreover, considering the potent pro-inflammatory and immunomodulatory properties of MIF, the anti-inflammatory effects of HDIs may also result, at least in part, from their ability to down-regulate MIF expression.

P215

Inhibitors of histone deacetylases impair innate immune responses of macrophages

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Background: Histone deacetylases are regulators of chromatin structure and gene expression. Originally investigated as anticancer drugs, histone deacetylases inhibitors (HDI) have emerged to be potent anti-inflammatory agents for auto-immune, allergic and inflammatory diseases. Yet, the influence of HDIs on innate immune responses remains scarcely studied.
Objective: To investigate the influence of HDIs on the generation of cytokines and reactive oxygen and nitrogen species (ROS et NO), the phagocytosis and the killing by murine bone-marrow derived macrophages (BMDMs) exposed to microbial products.
Methods: BMDMs were pre-incubated (1, 4, 8 or 14 h) with the HDIs trichostatin A (TSA), sodium butyrate (NaB) or valproic acid (VPA)

before exposure to LPS, Pam3CSK4, IFNg, LPS+IFNg, E. coli, S. aureus and phorbol myristate acetate (PMA). Cytokines, ROS and NO were measured by bioassay and ELISA and using DCFDA and the Griess reagent, respectively. Gene expression was analyzed using Agilent high density DNA arrays and confirmed for selected genes by real-time PCR. Inducible NO synthase (iNOS) protein levels were quantified by Western blotting.
E. coli and S. aureus phagocytosis and killing were quantified by conventional methods.
Results: TSA, NaB and VPA dose-dependently inhibited TNF, IL-6 and IL-12 release by BMDMs exposed to bacterial products. HDIs also reduced ROS production induced by PMA and NO production induced by PMA, LPS, LPS+IFNg and bacteria. In agreement with these observations, HDIs strongly hampered basal expression and/or stimulus-induced upregulation of p22, p40, p47, p67 and p91 phox mRNA and iNOS mRNA and protein expression in BMDMs. Finally, the phagocytosis and the killing of E. coli and S. aureus were strongly reduced in BMDMs pre-incubated with TSA and VPA.
Conclusions: HDIs exhibit profound inhibitory effects on innate immune functions of macrophages. These results suggest that HDIs might impair innate immune defenses and thus predispose patients, especially immunocompromised patients, who are treated with these agent to severe infections.

P216

Proteasome inhibitor bortezomib overrides TGF beta effect in human fibroblasts, imposing its anti-fibrotic action

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Background: Extracellular matrix (ECM) provides a controlled environment for cellular differentiation and tissue development. Its integrity is maintained through a balance between ECM components deposition and degradation. Excessive ECM breakdown is associated with rheumatoid arthritis, osteoarthritis, periodontitis and skin aging, as well as tumor invasion and metastasis. Excessive ECM deposition occurs in fibrotic diseases. One of the predominant ECM components found in fibrotic lesions is type I collagen and is composed of one alpha2 (COL1A2) and two alpha1 (COL1A1) subunits. ECM accumulation is controlled by relative expression levels of collagen, ECM degrading enzymes such as matrix metalloproteinases (MMPs) and their inhibitors, named Tissue Inhibitor of MMPs (TIMPs). We previously published that bortezomib, a proteasome inhibitor, exerts an in vitro anti-fibrotic activity, dominant over the pro-fibrotic phenotype induced by TGFbeta. We report here an extensive study of the transcriptional regulation of ECM genes in human dermal fibroblasts.
Methods: Variation in ECM mRNA and protein levels was determined by RT-PCR, enzyme-linked immunosorbent assay (ELISA) and Western blotting. Promoter activity of COL1A1 and MMP-1 genes was measured by reporter gene assay. Increase in binding of various transcription factors to specific promoter region of ECM genes was performed in vivo via chromatin immunoprecipitation (ChIP) and in vitro via electrophoretic mobility shift assay (EMSA).
Results: Bortezomib activated transcription of MMP-1 via increased binding to AP-1 site. Analogous response to bortezomib treatment was observed for MMP-13, whereas MMP-2 and MMP-9 were not affected. TGFbeta activated transcription of COL1A1 or COL1A2 via increased binding to AP-2 or SP1 sites, respectively. While bortezomib did not affect TGFbeta-induced binding of AP-2 to COL1A1 promoter, it completely abolished TGFbeta-induced binding of SP1 to COL1A2 promoter.
Conclusion: We identified elements of MMP-1 and COL1A1 promoters in fibroblasts, essential for bortezomib- or TGFbeta-mediated activation. Bortezomib treatment triggers converging signals: activation of MMP-1 and MMP-13 transcription, due to increased occupancy of AP-1 site and repression of TGFbeta-mediated induction of COL1A2 transcription on the SP1 site. These signals result in an anti-fibrotic phenotype in human fibroblasts.

P217

Limited influence of individual cytokines for vaccine-induced CD8+ T-cell response

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In the present study, we assessed the influence of cytokines on the induction of a vaccine induced CD8+ T cell response. So far, most studies addressing the role of cytokines for CD8-responses used viral models. These studies are hampered by the complexity of events triggered by viral replication, including the activation of a multitude of innate stimuli, owing to the complex structure of microorganisms that signal via different pathogen association molecular patterns (PAMs) and massive cell death usually occurring during infection.
Virus-like particles (VLPs) consist of multiple copies of certain structural viral proteins, which self-reassemble into spherical structures resulting in a viral shell devoid of genetic information required for viral replication. Vaccines on the basis of VLPs are very efficient in inducing CD8+ T cell responses when administered together with toll-like receptor-ligands, which are effective activators of APC. We were able to dissect the role of cytokines for the support of antigen-specific T cell expansion in the context of immuization with VLPs alone or VLPs applied together with distinct TLR stimuli by using mice deficient for Th1 promoting cytokines or their respective receptors.
To our surprise, the only cytokines that played a major role in CD8+ T cell-activation, expansion and memory establishment were type I interferons. Defective signalling in IL-12, IL-23 or IFNg had no major effect on establishment of functional CD8-responses and might therefore be considered redundant for the initiation of CD8+ responses.

P218

Polyclonal and specific antibodies mediate protective immunity against enteric helminth infection

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Helminths represent an evolutionary ancient species that have co-evolved with, and possibly asserted evolutionary pressure upon, the mammalian immune system. The enteric helminth Heligmosomoides polygyrus (Hp) is a natural murine parasite that establishes a chronic infection in wildtype mice. Anti-helminth immunity has long been recognized to involve CD4 T cells, yet the precise effector mechanisms responsible for parasite killing or expulsion remain elusive. We now show an essential role for antibodies in mediating immunity against H. polygyrus infection. The large majority of antibodies produced following H. polygyrus infection were polyclonal, associated with a robust extra-follicular plasma cell response, and functioned to limit egg production by adult parasites. Comparatively, affinity matured parasite-specific antibodies were required to prevent adult worm development, although these only developed after multiple infections. These data reveal previously unrecognized complementary roles for polyclonal and affinity matured parasite-specific antibodies in providing protection against enteric helminth infection.

P219

Ex-vivo cytotoxic function by influenza specific CD8 effector T-cells from healthy humans in memory phase

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Most healthy adults have long lasting influenza specific memory CD8 T cells in peripheral blood, primed by previous infection with influenza viruses. Relatively high frequencies of memory T cells with increased proliferative capacity form the basis for enhanced and accelerated protection upon re-infection. Memory cells are characterized by frequent expression of the IL-7 receptor CD127 and the costimulatory molecules CD28 and CD27. This is the case for the majority of influenza specific T cells in healthy adults, but other subsets of influenza specific T cells have different phenotypes and unknown functions. Here we show that up to ~20% of HLA-A*0201 / influenza matrix protein58-66 specific T cells from healthy donors did not express CD127 and/or CD28. In contrast to the majority of CD28pos cells, granzyme B and perforin were frequently expressed by CD28neg cells, suggesting that they may be effector cells. With a newly developed flow cytometry-based cytotoxicity assay we show

that these cells are indeed capable to lyse target cells in an antigen specific manner, directly ex vivo without prior stimulation, demonstrating the existence of influenza specific effector T cells in healthy humans. Sequencing of TCR alpha- and beta-chains revealed that the response to this epitope was dominated by ~1-3 T cell clonotypes. Interestingly, identical clonotypes were found as memory and effector cells, demonstrating for the first time that T cell memory is maintained by clonotypes that persist simultaneously in multiple differentiation stages. The data suggest that efficient secondary (memory) responses against influenza virus may depend on both, rapid expansion of memory cells and immediate function by long term persisting cytotoxic effector cells. Finally, these results reveal a surprising capacity of the human immune system for long term maintenance of circulating effector cells in absence of antigen, a property that deserves further investigation since it may be exploited for novel immunotherapy of minimal residual diseases.

P220

Role of HVEM in intestinal inflammation

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Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract encompassing two types of pathology; Crohn's disease and ulcerative colitis. Activation of the immune cells responsible for driving inflammation involves multiple signals and an improved understanding of how this process is regulated is key to the development of improved therapeutics. Stimulatory interactions between the recently identified TNF superfamily members, Herpes virus mediator (HVEM) and TNFS14 (LIGHT) have been implicated in the pathogenesis of IBD. However, conclusive evidence of their involvement is lacking. We used mice with a genetic deficiency in HVEM or LIGHT to investigate the impact of these molecules on experimental IBD. The experimental models employed were chosen to model distinct characteristics of the disease process. These were; i) chemical-induced inflammation that can be mediated by innate immune cells alone and ii) inflammation initiated by CD4+CD45RBhigh T cells following their transfer into immuno-deficient hosts. Our experiments showed that stimulatory HVEM interactions are required for the full activation of both innate cells and pathogenic CD4+ T cells during experimental IBD. These data establish LIGHT-HVEM interactions as a key stimulatory pathway in the initiation of intestinal inflammation and indicate that targeting this pathway may represent a novel means for the control of pathogenic immune responses causing IBD in humans.

P221

Decreased dendritic cells function and increased TGF-b production and intratumoral Foxp3+ regulatory T-cell numbers in intracerebral versus subcutaneous glioma correlate with site-specific immune surveillance

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Glioma are among the most fatal tumors. This has been attributed to immunosuppressive features of both the tumor and the CNS. However, the relative contribution of either the glioma or its localization has not been investigated. We report here that syngeneic GL261 glioma trigger a protective immune response only when growing subcutaneously, despite the fact that also intracerebrally grown gliomas are infiltrated by DC and T cells. This failure to control intracerebral gliomas correlates with increased immunosuppressive conditions in intracerebral tumors: tumor infiltrating dendritic cells from intracerebral gliomas are not able to stimulate T cell proliferation in vitro; brain-localized GL261 gliomas are characterized by significantly higher numbers of Foxp3+ regulatory T cells and higher expression of TGF-b1 and TGF-b2 mRNA when compared to GL261 gliomas in the skin. Thus, our data show that not the tumor but its localization dictates the efficiency of the anti-tumor immune response.

P222

IL-7 therapy improves long-term tumour antigen-specific CD8 T-cell responses following immunization with lentiviral vectors

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To improve the efficacy of anti-tumor T cell vaccination, we have produced lentivector-based vaccines. We showed that, in HLA-A2 transgenic mice, a single administration of recombinant lentivectors (rec. lv) encoding the melanoma-associated antigen Melan-A(26-35) elicited ex vivo-detectable antigen-specific effector CD8 T cells. This CD8 T cell response was sustained for a significantly longer period than the one elicited by peptide-based vaccines. Interestingly, we observed that Melan-A-specific CD8 T cells induced by rec. lv differed from those induced by peptides in that a majority of them expressed the memory marker CD127 (IL-7Ralpha) already at the peak of the response, and at every time point analyzed. We therefore asked whether IL-7R expression did confer an advantage in selecting the long-lived memory T cell sub-population among effector T cells. We observed that, at the peak of the response, there was no correlation between the expression of IL-7Ralpha and that of the survival molecule Bcl-2 on antigen-specific CD8 T cells. In contrast, after the contraction phase, cells expressing higher levels of IL-7Ralpha showed higher Bcl-2 expression. This suggested that the amount of IL-7 could be limiting during the expansion phase of the antigen-specific CD8 T cell response. To test this we administered human recombinant IL-7 (rIL-7) during immunization with rec.lv. We observed that administration of rIL-7 induced a strong expansion of both antigen-specific and total CD8 T cells as well as an up-regulation of Bcl-2. Moreover, mice that had received rIL-7 showed a significantly higher number of antigen-specific memory CD8 T cells compared to untreated mice. Altogether these data show that administration of IL-7 improves long-term tumor antigen-specific CD8 T cell responses following vaccination with lentiviral vectors.
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P223

Repetitive pertussis toxin administration protects against experimental autoimmune encephalitis

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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 IFN-g 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.

P224

Auto-reconstitution of the T-cell compartment by radio-resistant host-derived T-cells following lethal irradiation and bone marrow transplantation

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In man, bone marrow transplantation (BMT) with syngeneic or allogeneic BM is frequently used and shows curative results for a variety of haematological disorders such as malignant or genetic-based immune deficiencies. However, the restoration of an adaptive immune system in transplanted patients is a very slow process and during the initial reconstitution phase, lymphopenic patients lack adequate T cell-mediated immunity and often succumb to one of

several life-threatening infections and especially cytomegalovirus (CMV) infections. However, Roosnek and colleagues showed that the presence of residual host-derived T cells could provide efficient anti-viral immunity and help patients combat CMV infections in the lymphopenic period following BMT. The object of the current study was to further characterize host-derived T cell development and function in BMT mouse models. To this end, we generated chimeras by reconstituting lethally irradiated C57BL/6 mice with either syngeneic Rag2-deficient or CD3-epsilon-deficient BM. In such chimeras, donor-derived BM progenitors are not able to generate T cells and surviving T cells will be exclusively host-derived. Unlike previous studies, we found that host-derived thymopoiesis was initiated by DN1-2 prothymocytes having a conventional CD44+, CD117+, CD25-/+ phenotype and their differentiation recapitulated normal thymic ontogeny. Additionally, by comparing host-derived T cell numbers in thymus-bearing versus thymectomized hosts, we observed that the differentiation of host-derived thymocytes provided an important cohort of naïve, functional, mature T cells having a large TCR repertoire accounting for up to 35% the total T cell numbers in control chimeric or unmanipulated mice. These host-derived T cells might provide a first line of defence against infections during recovery from lymphopenia after BMT.

P225

STAT4 is required for maximal donor CD8+ CTL activity against host lymphohaematopoietic cells during acute graft-versus-host disease

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Acute graft-vs.-host disease impairs the nascent lymphohematopoietic system in recipients of allogeneic hematopoietic stem cell transplantation through yet to be defined mechanisms. Here, we demonstrate that the failure to engage signal transducer and activator of transcription (STAT)4 in donor T-cells prevented the development of maximal anti-host lymphohematopoietic cell cytotoxicity. While STAT4-deficient T-cells expanded normally in hosts, secreted both Interleukin-2 and Interferon-gamma and were able to induce hepatic GVHD, they did not impair host thymic T-cell development and splenic B-cell numbers. This defect was linked to a selective loss of perforin production and thus a defect in cytotoxic activity in response to MHC-disparate alloantigens. Consequently, strategies that interfere with the STAT4 signaling pathway in donor T-cells may limit damage to the recipient's lymphohematopoietic system and hence post-transplantation immunodeficiency.

P226

Protective anti-mycobacterial T-cell responses through in vivo activation of vaccine-targeted dendritic cells

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Vaccine efficacy 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. Using a promising candidate vaccine against tuberculosis (Ag85B-ESAT-6) formulated in the potent IC31 adjuvant, DC targeting and activation was studied in vivo, 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 IFN-g-mediated Ag85B-ESAT-6/IC31 responses were associated to 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, CD40 and IL-12p40 expression, was only identified when focusing on adjuvant- or antigen-labeled CD11c+ DCs, which were found to support T cell proliferation. Immunization with alum resulted into a similar proportion of antigen-associated DCs but without detectable enhancement of CD80, CD86, CD40 or IL-12p40 expression. Thus, potent protective IFN-g producing responses may be elicited by the exquisite activation of a minute number of in vivo targeted DCs.

P227

Contribution of CD8+ T-cell subpopulations in peripheral reconstitution following lymphopenia

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Introduction: T cell reconstitution occurs following lymphopenia even in the absence of thymic output. We are investigating how the mechanisms of recovery and the number and composition of the initial T cell pool impact on the phenotype and function of T cells following lymphopenic-driven expansion.
Methods: Naive, central-memory (TCM) or effector-memory (TEM) CD8+ T cells were sorted from non-manipulated donors and adoptively transferred into T cell deficient hosts (CD3-/-) or into WT hosts after total body irradiation. Donor CD8+ T cells from each subpopulation and host cells could be distinguished through the expression of different combinations of congenic markers (Ly5 or Thy1). Expansion and differentiation of CD8+ T cell progeny was evaluated by FACS at different time-points and using Affymetrix genechip technology.
Results: We found that cells from each subset were able to expand and contribute to the recovered CD8+ T cell pool. TCM progeny were the most represented in the recovered T cell pool, both in CD3-/- hosts and especially in irradiated hosts. The kinetics of expansion was subset-dependent, with TEM CD8+ T cells expanding more rapidly than TCM and naive T cells. The phenotype of the recovered CD8+ T cells revealed that while the progeny of naive T cells acquired a TCM or TEM phenotype, the progeny of TCM and TEM maintained to a large extent the original phenotype with a small proportion of TEM converting to TCM. We are currently investigating whether these findings could be extended using gene expression profiling studies. We also found that expansion potentials and differentiation were dependent on the presence of other CD4+ or CD8+ T cells. Indeed, in the presence of CD8+ T cells the TEM->TCM conversion rate was decreased whereas in the presence of naive CD4+ T cells CD8+ T cell recovery and differentiation increased dramatically, and in the presence of CD4+CD25+ Tregs expansion and differentiation of nave CD8+ T cells was greatly impaired.
Conclusions: The reconstitution of peripheral CD8+ T lymphocyte numbers relies on the expansion of lymphocytes from different subsets. However, the contribution of each subset is dependent from its relative representation at the onset of lymphopenia and from the composition of the remaining lymphocyte pool.

P228

Expression and function of chemokine receptors on mouse TH17 cells

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Different types of CD4+T helper (TH) cells develop from naive T cells under the influence of polarizing signals and master transcription factors. Recently a new subset of T cells, distinct from TH1 and TH2, producing IL-17 (TH17) was identified. IL-17 is a cytokine that induces production of chemokines and antimicrobial peptides by tissue cells leading to recruitment of neutrophils and inflammation. In addition TH17 lymphocytes appear to be essential in the pathogenesis of several inflammatory and autoimmune diseases. It has been shown that expression of the chemoattractant receptors CCR6 in humans identifies a population of memory and effector TH17 cells in both peripheral blood and inflamed tissues. In order to evaluate the expression and role of chemokine receptors on mouse IL-17 producing T cells, we isolated CD44lo CD62Lhi naïve CD4+ and CD8+ T cells from spleen and lymph node of C57/B6 mice and primed these cells in vitro with DCs, stimulated with either LPS, CpG or Zymosan, in the presence of anti-CD3 antibodies and TGF-beta. A sizable proportion of both CD4+ and CD8+ T cells activated under these stimulatory conditions differentiated into IL-17 producing T cells and expressed CCR6. When CD4+ and CD8+ T cells were sorted according to CCR6 expression, IL-17 release and expression of RORgammat, the master transcription factor required for mouse TH17 differentiation, were found almost entirely restricted to CCR6+ population. Work is progress aims at defining the role of CCR6 in directing migration of TH17 cells at sites of inflammation or infection.

P229

Anti-nucleosome antibodies as a marker of active proliferative lupus nephritis

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Objective: Anti-nucleosome autoantibodies were previously described to be a marker of active lupus nephritis. However, the true prevalence of anti-nucleosome antibodies at the time of active proliferative lupus nephritis has not yet been well established. Therefore the aim of this study was to define the prevalence and diagnostic value of autoantibodies against nucleosomes as a marker for active proliferative lupus nephritis.
Methods: In a prospective multicenter study, anti-nucleosome and anti-dsDNA antibodies were determined in 35 adult SLE patients at the time of the renal biopsy demonstrating class III or IV lupus nephritis and compared to 59 control SLE patients.
Results: Elevated concentrations of anti-nucleosome antibodies were found in 31/35 (89%) patients with active proliferative lupus nephritis compared to 47/59 (80%) control SLE patients. No significant difference between the two groups with regard to the number of positive patients ($p = 0.2$) or the antibody concentrations ($p = 0.2$) could be found. The area under the receiver-operator characteristic (ROC) curve as a marker of the accuracy of the test in discriminating between proliferative lupus nephritis and inactive/no nephritis in SLE was 0.581 (CI 0.47–0.70, $p = 0.2$).
Conclusion: Anti-nucleosome antibodies have a high prevalence in patients with severe lupus nephritis. However, our data suggest that determining anti-nucleosome antibodies is of limited help in the distinction of patients with active proliferative lupus nephritis from SLE patients without active renal disease.

P230

Increased numbers of circulating polyfunctional Th17 memory cells in patients with seronegative spondyloarthropathies

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Reassessment of autoimmune inflammatory disorders in mouse models has suggested that among the major factors involved in their pathogenesis the IL-23/IL-17 axis, rather than the previously postulated IL-12/IFNgamma axis plays a fundamental role. Indeed, the presence of IL-17 producing CD4+ T cells (Th17 cells) has been shown to be necessary for the induction of different autoimmune diseases in mouse models. However, the implication of this newly identified CD4+ T cell population in mediating autoimmune pathologies in humans has not yet been demonstrated. In this study we measured the levels of circulating Th17 cells in several human autoimmune pathologies, as compared to healthy donors. We document elevated levels of circulating Th17 cells in psoriatic arthritis as well as ankylosing spondylarthritis patients, but not in peripheral blood of rheumatoid arthritis, vitiligo and melanoma patients. In addition, a more pronounced differentiation state and poly functionality of Th17 was observed exclusively in arthritis patients. Unexpectedly, we found a population of CCR6- Th17 cells in psoriatic arthritis as well as ankylosing spondylarthritis patients, while this subset was absent in HDs and rheumatoid arthritis patients, suggesting a possible implication in disease establishment. Finally, the increase in Th17 cells did not correlate with a decrease neither in frequencies, nor in function and suppressive activity of circulating regulatory T cells. Together, these observations suggest a possible role for Th17 cells in the pathogenesis in the seronegative subset of inflammatory arthropathies.

P231

Analysis of naturally acquired CD4+ T-cell responses to MAGE-A3 and MAGE-A4 cancer/testis antigens in patients with resected head and neck squamous cell carcinoma

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Despite advances in the medical and surgical treatment of Head and Neck (HN) squamous cell carcinoma (HNSCC), long term survival has remained unchanged in the last 20 years. The obvious limitations of traditional therapeutic options strongly urge the development of novel therapeutic approaches. The molecular cloning of tumor antigens recognized by T lymphocytes in recent years has provided targets for specific immunotherapy. In this regard, frequent expression of Cancer Testis Antigens (CTA) has been repeatedly observed among HN tumors. We analyzed CTA expression in 46 HNSCC patients and found that MAGE-A3 and/or -A4 CTA were positive in over 70% of samples, regardless of the anatomical site of primary tumors in the upper aerodigestive tract. Still, immune responses against these CTA in HNSCC patients have not yet been investigated in detail. In this study we assessed the responsiveness of HNSCC patient's lymphocytes against overlapping peptides spanning the entire MAGE-A3 and -A4 proteins. After depletion of CD4+CD25+ regulatory T cells, and following three rounds of in vitro stimulation with pools of overlapping peptides, peripheral blood mononuclear cells (PBMCs) of HNSCC patients were screened by IFN-g and TNF-a intracellular cytokine staining for reactivity against MAGE-A3 or -A4 derived peptides. Cytokine secreting CD4+ T cells, specific for several peptides, were detected in 7/7 patients. In contrast, only 2/5 PBMC from healthy donors showed weak T cell responses against 2 peptides. CD4+ T cells specific for one epitope MAGE-A3(281-295), previously described as an HLA-DR11 restricted epitope naturally processed and presented by dendritic cells and tumor cells, were detected in two patients. MAGE-A3(161-175) specific CD4+ T cells were found in one patient. Six MAGE-A3 and -A4 new epitopes are being characterized. Together, these data suggest that naturally acquired CD4+ T cell responses against CT antigens occur in vivo in HNSCC patients, providing a rational basis for the use of the identified peptides in vaccination protocols.

P232

Strong Epstein-Barr-Virus-specific CD8+ T-cell response in patients with early multiple sclerosis

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Introduction: Epstein-Barr virus (EBV), in contrast to cytomegalovirus (CMV) has been repeatedly associated with a higher relative risk of developing multiple sclerosis (MS). Here, we studied the EBV- and CMV-specific T cell responses in patients with MS, other neurological diseases (OND) as well as in healthy subjects (HC).
Methods: We enrolled patients with inflammatory MS, chronic MS, OND and HC and analyzed the EBV- and CMV-specific T cell responses by ELISPOT (IFN-gamma secretion).
Results: The EBV-specific CD4+ T cell responses were similar between the groups, however we found that EBV-specific CD8+ T cells in inflammatory MS patients secreted a higher amount of IFN-gamma than all other categories, including chronic MS patients. Interestingly, we found that the shorter the interval between MS onset and the assay, the higher the intensity of IFN-gamma secreting EBV-specific CD8+ T cells. However, the disease activity played no role as we did not find differences between MS patients in relapse or in remission.
By contrast, CMV-specific CD4+ T cell responses were moderately enhanced in patients with inflammatory and chronic MS as compared to OND and HC subjects. No difference was found in the CMV-specific CD8+ T cell responses and there was no correlation with the time elapsed since MS onset.
Conclusion: Our data suggest that EBV, more than CMV, might be involved in the onset of MS and that this effect might be mediated by CD8+ T cells.

P233

Giant cell arteritis with large vessel impairement

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Background: Giant cell arteritis (GCA) is the most common vasculitis in adult. It involves large and medium-sized vessels, rarely occurs before age 50 and predominates in women. Involvement of the aorta is not rare, but often unrecognized because asymptomatic at the beginning.
Findings: We report the case of a 76-year old patient with a past history of diabetes, hypertension and hyperlipidemia, who presented with chest pain and shortness of breath since several months, weight loss (6 kg) and loss of appetite over one year, marked fatigue and fever. ESR fluctuated in the range of 50 to 97 mm/h and CRP between 50 and 100 mg/l. There was a discrete anemia (Hb 100 g/l) and slightly positive antinuclear antibodies (homogenous, 1/80). Echocardiography showed a massive infiltration of the aortic valve and the ascending aorta. CT-scan revealed a small dissection of the ascending aorta and a thick infiltrating tissue surrounding the aorta, the pulmonary artery, the upper portion of the right ventricle and the aortic valve. Aortic valve and ascending aorta were immediately replaced. Histologic analysis of ascending aorta and aortic valve displayed an important fibrotic infiltrate with granulomatous inflammation, multinucleated giant cells and necrosis. On the basis of clinical features, laboratory and radiologic findings as well as histology, we retained the diagnosis of GCA. Despite steroid treatment with prednisone (1 mg/kg) and cyclophosphamide, inflammation progressed and led to severe aortic insufficiency and mesenteric ischemia. The patient died 6 months after diagnosis in the context of sepsis, after a second operation for severe aortic insufficiency.
Conclusions: Glucocorticoids remain the mainstay of therapy in GCA (1 mg/kg/day at the beginning). Their introduction has to be prompt, followed by tapering on a minimum of 1–2 years (relapses >60% of cases). The use of other immunosuppressor drugs (azathioprine, MTX, anti-TNF) has not been validated yet in large, randomized, placebo-controlled studies. The mortality of GCA is comparable to that of the general population, except for patients with thoracic aorta dissection for whom the average survival is 1.1 yr. Early detection by CT-scan, MRI or PET-scan is largely indicated in patients with a suggestive history (elevated ESR and CRP, thoracic pain, dyspnea, hypotension, aortic valve insufficiency).

P234

Detection of IgG isotypes using designed ankyrin repeat proteins

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Background: IgG subclass distribution is a characteristic of an antibody response in various diseases and is of pathological relevance. To investigate the association of selective IgG subclass deficiencies to some diseases, binders capable of distinguishing the distinct subclasses are required. Traditional approaches using monoclonal antibodies obtained by animal immunization resulted in few specific binders showing assay restrictions and light chain or allotype bias. Therefore there is interest in finding alternatives to monoclonal antibodies with better specificity. Recently, a novel type of binding molecules based on repeat units named designed ankyrin repeat proteins (DARPins) has been proposed as alternatives to antibodies. They are built from 33 amino acid repeat units that stack together to form a hydrophobic core and a large solvent accessible surface that is involved in target binding. Using a consensus design strategy, DARPin libraries of varying repeat numbers and randomized surface residues have been generated.
Methods: Using ribosome display, we selected binders against the constant heavy chain region of IgG1 from two combinatorial libraries containing either two or three repeat modules. After production and affinity purification on metal chromatography, binders were tested for their reactivity on different immunoglobulin isotypes by ELISA and their specificity was compared with commercial anti-IgG1 antibody.
Results: Three DARPins were obtained which recognized specifically IgGs and showed no reactivity with other immunoglobulin isotypes. Their binding characteristics were comparable to those of a commercial anti-IgG1 antibody, namely they recognized IgG1 and in lesser extent also IgG4 but did not cross-react with IgG2 and IgG3 subclasses. Furthermore their binding on IgG1 and IgG4 were concentration dependent with a higher response to IgG1 than to IgG4 at the same concentration.
Conclusion: With these experiments we demonstrated the possibility to rapidly select specific binders from DARPin libraries recognizing IgG subclasses analogous to a commercial antibody. Currently we investigate whether DARPins can be isolated that recognize specifically IgG subclasses.

P235

Alefacept therapy improves atopic eczema

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Background: Atopic dermatitis (AD) is a chronic inflammatory skin disease, orchestrated by T cells, dendritic cells, eosinophils, B cells, mast cells, and keratinocytes. The fusion protein alefacept (anti-LFA3) decreases the activation of memory-effector T cells by blocking costimulation and by reducing T cell numbers. This study aimed to prove the efficacy of alefacept in AD and to investigate histopathologic and immunologic parameters.
Methods: Ten patients with moderate to severe AD were treated with 15 mg alefacept (Amevive®, Biogen) I.M. weekly over 12 weeks. Peripheral blood cell analysis including immunophenotyping, clinical parameters (EASI, pruritus score, concomitant medication), skin histology, immunofluorescence analysis of skin infiltrating cells, cytokine expression by PBMC and by skin infiltrating cells on mRNA and protein levels were monitored.
Results: All patients showed an improvement starting between 6 to 8 weeks after initiating therapy. The EASI significantly decreased (18.7 ± 1.9 at week 0 versus 4.7 ± 1.6 at week 12; $p < 0.001$) paralleled by a reduction of pruritus. This effect lasted over the study period of 24 weeks (EASI 2.8 ± 0.7). Skin biopsies revealed a significant reduction of spongiosis, acanthosis and dermal infiltrate. The number of cytokine expressing CD4+ and CD8+ cells, but also B cells, eosinophils was markedly reduced after therapy.
Conclusion: Alefacept is effective in the treatment of AD by reducing skin inflammation. Alefacept may present a new therapeutic tool in patients refractory to topical treatment.

P236

Prevalence and significance of anti-HLA antibodies after liver transplantation

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Background: The pathogenic role of anti-HLA antibodies (AHA) after kidney transplantation is well established. However, its significance after liver transplantation remains unclear. The aim of our study was to determine the prevalence and significance of AHA after liver transplantation.
Methods: Between January 2007 and November 2007, all liver transplant recipients who were greater than 6 months post-transplantation and followed regularly at our transplant outpatient clinic (n = 95) were screened for AHA. All clinical and electronic records were reviewed. Serum samples were tested using multiplex technology (Luminex). A liver biopsy had been performed in 55 out of the 95 patients based on clinical grounds but no routine protocol biopsies were performed. Immunosuppression was calcineurin inhibitor-based in 90 patients, sirolimus-based in 4 patients and one patient had no anti-rejection therapy (operationally tolerant recipient).
Results: The mean time from transplantation to study was 85 months (range 6–248 months). Overall, AHA were found in 23/95 (24.2%) of patients (5 had anti-class I alone, 13 anti-class II alone, and 4 had both anti-class I and II). However, only 4/95 patients (4.2%) had donor-specific antibodies (DSA) (one anti-class I and 3 anti-class II). Twenty-one out of 95 patients (22.1%) had a history of past or current biopsy-proven or radiological biliary complications (chronic rejection, ischemic cholangitis, ischemic type biliary lesions or biliary anastomosis stricture). Among patients with AHA, 4/23 (17,4%) had biliary complications, while it was 17/72 (23.6%) in patients without AHA (NS). Among patients with DSA, 3/4 (75%) had biliary complications (two with biopsy-proven chronic rejection in association with biliary strictures and one with ischemic cholangitis following hepatic artery thrombosis), versus 1/19 (5.3%) patients with AHA but no DSA ($p = 0.009$), versus 16/72 (22.2%) patients without AHA ($p = 0.046$). In patients with DSA, immunosuppression was not different than in patients without DSA.
Conclusions: We found a 24% AHA prevalence. The presence of DSA, but not of AHA, was significantly associated with an increased incidence of biliary complications including chronic liver allograft

rejection. The exact mechanisms and possible causal relationship linking DSA to biliary complications remain to be studied. Larger prospective trials are thus needed to further define the role of AHA and in particular of DSA after liver transplantation.

P237

Evaluation of combined long synthetic peptides (LSPs) of MSP2 as a malaria candidate vaccine

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Merozoite surface protein 2 (MSP2) is a promising malaria candidate vaccine against the Plasmodium falciparum infection. The recombinant protein of MSP2 based on the 3D7 sequence was found to be safe and immunogenic as part of the Combination B clinical trial. Also observed were marked reduction in level of parasitemia and allele-specific protection as the Pf parasites found in the infected volunteers were mainly of the FC27-allelic family. It is thus proposed that a combination of both allelic families will further improve the efficacy of the vaccine.
An alternative approach to the production of antigens as recombinant protein is peptide synthesis. This has been found to reduce the production time and thus shorten the preclinical evaluation period of candidate vaccines.
We present the results of the evaluation of combined LSPs corresponding to the two allelic families of MSP2 as malaria candidate vaccine. Both LSPs were well recognized by sera of individuals living in malaria-endemic regions and peptide-specific antibodies were associated with protection against clinical malaria. Affinity- purified human antibodies recognized native antigens in infected erythrocytes and were also active in antibody dependent cellular inhibition (ADCI) assays. The LSPs also induced high antibody titers in different strains of mice.
These results justify further development of the MSP2 LSPs as malaria candidate vaccine.

P238

Treatment with rituximab of cryoglobulinemic vasculitis, which persisted despite successful treatment of HCV with antiviral treatment

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Background: Cryoglobulinemia is the most frequent extrahepatic manifestation of chronic hepatitis C (HCV). Cryglobulinemia can lead to vasculitis in target organs such as the skin, joints, peripheral nerves and the kidney. Its therapy is not yet fully defined, although anti-viral therapy often eradicates cryoglobulins and improves vasculitis. The place for anti-CD20 monoclonal antibody rituximab therapy is still an ultimate choice in case of failure of more classical approaches.
Case report: A 75-year old man was admitted to our hospital with multiples ulcers of both legs, resistant to medical and surgical treatment (graft) since 12 months. A cutaneous biopsy revealed leucocytoclastic vasculitis. Work-up of the etiology of this leucocytoclastic vasculitis showed type III cryoglobulinemia in relation with a hepatitis C infection of genotype 1b. Treatment with prednisone was unsuccessful. We began a treatment with peginterferon alpha-2a and ribavirin which permitted rapid resolution of ulcers but which had to be stopped after 8 months because of extreme fatigue. Despite sustained viral response (the patient is still aviremic 2 years after antiviral treatment), type III cryoglobulinemia at a concentration of 1.25 g/l persisted. The patient developed, one year after stopping antiviral treatment, painful paresthesias and hyposensitivity of both legs with difficulty to walk in relationship with a severe, objectively demonstrated polyneuropathy. A biopsy of sural nerve showed an acute and chronic neuritis with perivascular infiltration, compatible with cryoglobulinemic vasculitis. We decided to administer a cycle of four weekly perfusions of rituximab (375 mg/m^2). Four months later pains and paresthesias of the legs disappeared. The concentration of cryoglobulinemia dropped from 0.39 g/l before rituximab treatment to 0.17 g/l after treatment.
Conclusions: Despite successful antiviral treatment of hepatitis C infection, cryoglobulins may persist and still cause clinically significant cryoglobulinemic vasculitis. Rituximab is a treatment option in cryoglobulinemic vasculitis, but the place of rituximab in treating hepatitis C associated cryoglobulinemic vasculitis warrants further investigation in randomized, placebo-controlled studies.

P239

Low levels of anti-phophorylcholine IgM are associated with more severe angiographic coronary artery disease in patients suffering from acute coronary syndrome and poorer clinical outcome at 6 months

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Background: Phophorylcholine (PC) is a major component in platelet activating factor and in oxidized low density lipoprotein (oxLDL) and is also the main epitope recognized by anti-pneumococcus antibodies. Antibodies of IgM subclass directed to PC (aPC IgM) have been shown to be a potential protective cardiovascular factor in animals but their role in humans is unclear. Our objective was to find out aPC IgM association with angiographic coronary artery disease (CAD) severity and clinical outcome in patients suffering from acute coronary syndrome (ACS).
Methods: We determined aPC IgM by ELISA on 125 consecutive ACS patients and assessed their association with angiographic CAD severity, NT-proBNP, oxidized LDL (oxLDL) levels, and outcome at 6 months. Adverse Composite Cardiovascular Outcome (ACCO) was defined by all cause mortality, acute coronary syndrome relapse, stroke, and acute heart failure needing hospitalisation.
Results: Patients with one or less CAD had significantly higher aPC IgM levels than patients with two or more CAD. Stepwise regression showed that aPC IgM level was an independent predictor of CAD severity and we found significant correlations between aPC IgM titres, numbers of CAD, oxLDL and NT-proBNP. aPC IgM levels below 17 U/ml were associated with a 3.1 relative risk increase for ACCO (95%CI: 1.23–7.75; p = 0.03) and 3.68 increased risk for death and myocardial infarction (95 %CI:1.5–8.88; p = 0.02) at 6 months. ROC curve analysis showed that anti-PC IgM was a significant discriminatory test to predict ACCO (AUC: 0.62;95%CI: 0.48-0.78, p = 0.04). At the cut-off of 17 U/ml, the sensitivity was 31.3%, specificity 89%, positive predictive value 29%, and negative predictive value was 89%.
Conclusion: Low anti-PC IgM level upon admission in ACS is an independent predictor of angiographic CAD severity and associated to poorer cardiovascular outcome at 6 months.

P240

Low risk of anti-HLA antibody sensitisation after combined kidney and islet transplantation

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Background: De novo anti-HLA antibody is a major problem after kidney transplantation leading to humoral rejection and a decrease in graft survival. Recent reports have suggested that islet transplantation alone is associated with a high rate of sensitization. The withdrawal of the immunosuppressive therapy due to the progressive non function of the islets could explain the high rate of sensitization. As the specific risk of immunization of multiple islet infusions remains unknown, we studied the immunization rate in our cohort of multiple islet infusions transplant recipients.
Method: De novo anti-HLA antibodies in 37 patients after islets alone (N = 8), islet-after-kidney (N = 13) and simultaneous islet-kidney (N = 16) transplantation were analyzed by ELISA and Luminex over time.
Results: The rate of immunization was 10.8 % which is comparable to the risk of immunization after kidney transplantation alone.
Conclusion: Multiple islet infusions do not represent a specific risk for the development of anti-HLA antibodies in our cohort.

P241

Examination of the inflammatory nature of different placental syncytiotrophoblast microparticles preparations on human monocytes

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Background: Preeclampsia is a placenta-dependent disorder specific to human pregnancies, which is hallmarked by hypertension and proteinuria in the second half of pregnancy. An excessive maternal inflammatory response and a dysfunctional maternal endothelium are thought to be at the basis of the clinical manifestations of preeclampsia. The placental epithelial layer, the syncytiotrophoblast, constantly sheds microparticles (STBM) into the maternal peripheral blood, which come in contact with maternal endothelial and immune cells. STBM are released in excessive amounts in preeclampsia. The aim of this study is the characterisation of the inflammatory properties of in vitro prepared STBM on maternal monocytes.
Materials and methods: STBM were generated by two different approaches: (1) placental villous explant cultures incubated at 20% O2 for 72 hours; (2) mechanical dissection of villous tissue. STBM were added to cultures of fresh human blood monocytes, prepared by Ficoll and subsequent negative MACS. In some cases NF-kappaB inhibitors were included. Cell viability was analysed by the WST-1 assay. Phenotypic analysis was performed by FACS and cytokine production was assayed by ELISA.
Results: Both STBM preparations did not affect cell viability. Treatment of monocytes with mechanical STBM resulted in a decreased CD54 fluorescent intensity, but did not increase cytokine release. STBM prepared from villous explant cultures enhanced CD54 fluorescent intensity on monocytes and increased IL-8 and IL-6 secretion in a dose-dependent manner. Villous STBM-induced cytokine secretion was reduced by treatment with NF-kappaB inhibitors.
Conclusions: Both STBM preparations have different effects on human blood monocytes. Mechanical STBM have minor impacts. Villous STBM induce a proinflammatory phenotype in fresh monocytes, partly mediated by NF-kappaB. Altered regulation of CD54 induced by STBM treatment may modulate adhesion properties of monocytes. Increased production of IL-8 and IL-6 could recruit further immune cells leading to generalized inflammation.

P242

Antibodies against mycobacteria may define a more severe Crohn's disease phenotype and are partly cross-reactive with yeast mannan but are unlikely to be responsible for the induction of anti-Saccharomyces cerevisiae seropositivity

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Background: A subgroup of patients with Crohn's disease (CD) develop anti-Saccharomyces cerevisiae antibodies (ASCA) directed against yeast cell wall mannan. The mechanism of ASCA generation is still unclear. Mannans also occur in mycobacteria as lipoarabinomannan (LAM).
Objective: To test whether an immune reaction against mycobacteria may play a role in ASCA generation, and whether it associates with a distinct disease phenotype.
Methods: Lysates were prepared from M. avium, M. smegmatis, M. chelonae, M. bovis BCG (BCG) and M. avium ssp. paratuberculosis (MAP) by sonication. ASCA, presence of the dominant ASCA epitope, and anti-mycobacterial IgG were determined by ELISA. Affinity purification of ASCA and anti-mycobacterial antibodies were performed to assess cross-reactivities. CD patients were grouped according to the Vienna criteria.
Results: The dominant ASCA epitope terminal alpha-1,3 linked mannose was found at different extents in mycobacterial lysates and was absent or almost absent in purified LAM and mannose-capped LAM (ManLAM). Fifteen to 45% of CD patients but only 0-6% of controls had IgG against different mycobacterial strains. Anti-mycobacterial IgG most significantly associated with ASCA-positive patients. ASCA-positivity and deficiency for mannan-binding lectin (MBL), which recognizes cell wall mannans, synergistically associated with anti-mycobacterial antibodies. Seropositivity ranged between 24% (purified LAM) and 71% (M. smegmatis) for ASCA-positive/MBL-negative CD patients, compared to 4% (LAM) to 26% (BCG) for the ASCA-negative/MBL-positive subgroup. In a subgroup of ASCA/anti-mycobacterial IgG double-positive patients, anti-mycobacterial antibodies represent cross-reactive ASCA. Vice-versa, the predominant fraction of ASCA did not cross-react with mycobacteria. Finally, a more severe disease phenotype significantly associated with elevated titers of anti-M. chelonae, anti-LAM and anti-ManLAM IgG ($p = 0.0401$, 0.0352 and 0.0209, respectively).
Conclusions: Similar to ASCA, seroreactivity against mycobacteria may define CD patients with a severe disease phenotype and with a predisposition to mount immune responses against ubiquitous antigens. While in some CD patients anti-mycobacterial antibodies to a certain extent cross-react with yeast mannan, these cross-reactive antibodies only represent a fraction of total ASCA. Thus, mycobacterial infection is unlikely to play a role in ASCA induction.

P243

ABO-incompatible living-donor kidney transplantation: immunoabsorption with Glycosorb® columns is not specific

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Introduction: ABO-incompatible living-donor kidney transplantation has become a valuable alternative to increase the donor pool. Pretransplant column immunoabsorption (IADS) is successfully used to prevent (hyper-) acute graft rejection by removing anti-blood group A/B antibodies (Ab) using synthetic blood-group antigens (Ag). However, there is little in vivo data available regarding the effect of IADS on total, on anti-blood group A/B and on anti-vaccine specific Ab levels.
Material and methods: Serum samples were collected from 17 patients before and after IADS using Glycosorb® ABO columns. Total IgM and IgG levels were measured by nephelometry, Ab levels against tetanus, diphtheria, pneumococcus and hemophilus Ag by ELISA. Anti-blood group A/B Ab were measured by agglutination, indirect antiglobulin test (IAT) and flow cytometry (ABO FACS).
Results: The effect of IADS was most prominent during the first session and a significant reduction of both, blood group A/B donor-specific Ab, as well as compatible blood group A/B Ab was demonstrated. The median reduction of donor-specific anti-A/B was 79% and 73% for IgM and IgG during the first and 28% and 29% during the second IADS, decreasing below 20% for further sessions. The reduction of compatible anti-A/B Ab during the first IADS session was 59% (23-87%) for IgM and 34% (8-76%) for IgG. IADS reduced all IgG subclasses without reaching statistical significance. Anti-vaccine Ab levels against carbohydrate Ag (hemophilus influenzae, pneumococcus) were significantly reduced after IADS without reduction of Ab levels against protein Ag (tetanus, diphtheria). Intravenous immunoglobulin (IVIG), administered before transplantation, increased the IgG anti-blood group A/B levels without an effect on agglutination titers. Moreover, IVIG increased anti-vaccine Ab levels against both carbohydrate and protein Ag. Rituximab®, administered 3 weeks before transplantation, did not reduce anti-A/B Ab levels before the start of IADS.
Conclusions: Glycosorb® ABO IADS was not specific for the synthetic blood group Ag present in the columns since total and carbohydrate anti-vaccine Ab levels were significantly reduced. In contrast, IADS does not seem to affect anti-protein Ab levels. The protective anti-vaccine Ab can be restored by the administration of IVIG. These findings allow a better understanding of the Ab kinetics following IADS and help to improve future protocols.

P244

Case report: an adolescent with a vasculitis, it is not always Henoch-Schönlein purpura

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Systemic vasculitides diagnosed in children are mainly Henoch-Schönlein purpura (HSP), Kawasaki Disease and post-infectious vasculitis. The more severe forms of vasculitides are very rare during childhood and adolescence, with an estimated prevalence of about 1 for 100'000 children.
We present the case of a 13 years old boy who presented with pain and swelling in the left ankle and with purpura on the adjacent skin. The laboratory showed proteinuria and microhematuria. The patient was diagnosed as HSP and received symptomatic treatment. Few days later, he developed bloody diarrhoea and abdominal pain. The abdominal ultrasound was normal, and he was treated with prednisone for a few days. Three weeks later, he presented diffused arthralgia, a painful and swollen calf, persistent abdominal pain, one

episode of hematemesis, and persistent hematuria and proteinuria. A Doppler ultrasound did not show thrombosis. He was then sent to the hospital for further investigations. The systematic anamnesis revealed asthenia and weight loss since a few months. He was also complaining about aphtous stomatitis, epistaxis, conjunctival hyperaemia, rash on the face and the elbows, headache and cervical pain. The laboratory showed systemic inflammation (ESR 47), normal blood count, normal liver, kidney and muscles values, elevated complement (C3 1,66g/l), normal antistreptolysin-O, negative antinuclear antibodies. C-ANCA were positive with elevated anti-proteinase3(147kU/l). The diagnosis of Wegener's Granulomatosis was suspected on the clinical and laboratory findings. It was confirmed by a CT scan showing maxillary and ethmoidal mucosal thickening and multiple small opacities on both lungs, and by the histopathology of the kidney and the intestinal mucosa.
Our patient was treated with high dose steroids to induce the remission and azathioprine to allow steroid weaning. The evolution was favourable with complete inactivity of the disease and disappearance of the c-ANCA, even with low doses of steroids.
The association of purpura, arthritis, proteinuria and abdominal pain is relatively common in childhood and suggests the diagnosis of HSP. Our case shows that children may develop other types of vasculitis which are less common and more severe. The physician should be aware of the differential diagnosis with other vasculitides, when the presentation or follow-up are not classical. Early diagnosis of Wegener granulomatosis is essential for the prognosis and to avoid complications.

P245

Evaluation of therapeutic effects of monoclonal antibodies, directed against cluster of differentiation expressed by B-chronic lymphocitic B-cell leukaemia, in vitro and in vivo

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New monoclonal antibodies (mAb) against different antigens expressed by B-CLL, developed within a European program, were evaluated.
Binding and internalisation assays were performed with 125I-radiolabelled antibodies on various cell lines including the original and the CD5-transfected leukaemia cell line JOK1 and JOK1-5.3, respectively. mAb mediated cell proliferation inhibition was tested with a radiolabelled-thymidine analogue and FACS analysis. Anti-tumour efficacy was tested in an intra-peritoneal (i.p.) JOK1-5.3 B-CLL model in SCID mice using early and delayed (1 and 7 days, respectively) antibody treatment initiation. Mice were sacrificed at appearance of significant disease.
Radiolabelled mAbs against CD5, CD32, CD71 and anti-HLA-DR showed specific direct bindings of ~45–70% on target cells and induced variable internalisation effects. Anti-HLA-DR strongly inhibited cellular proliferation of all cell lines tested and directly induced apoptosis. Anti-CD71 inhibited the proliferation of most cell lines with an accumulation of cells in early S-phase, but had no effect on JOK1-5.3 cell growth. No proliferation inhibition was observed with anti-CD5 or anti-CD32 mAbs. JOK1-5.3 tumour mice treated with anti-CD71 or anti-HLA-DR showed a prolonged survival of about 20 days compared with untreated or isotype matched irrelevant mAb treated controls. Similar results were observed after treatment with anti-CD5 as compared to untreated controls or sham-treated JOK1 tumour grafted mice (CD5-).
In conclusion, all mAbs inhibited JOK1-5.3 leukemia development while showing variable in vitro effects. The broader in vivo efficacy of certain mAbs might be explained by their IgG2 subclass, allowing recruitment of effector functions, but the in vivo efficacy of the anti-CD71 mAb, of mouse IgG1 subclass, and having no effect on JOK1 5.3 in vitro, remains currently unexplained.

P246

The presence of a CD4+ CD25high CD45RO+ CD127high T-cell population correlates with the clinical status of kidney transplant recipients

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Background: Recent data have suggested that a population of CD4+ CD25high T cells, phenotypically characterized by the expression of CD45RO and CD127, is significantly expanded in stable liver and kidney transplant recipients and represents alloreactive T cells. We analyzed this putative new alloreactive cellular marker in various groups of kidney transplant recipients.
Patients and methods: Flow cytometry was used to analyze the expression of CD25, CD45RO and CD127 on peripheral CD4+ T cells. Of 73 kidney recipients, 59 had a stable graft function under standard immunosuppressive therapy (IS), 5 had biopsy-proven chronic humoral rejection (CHR), 8 were stable under minimal IS and one was an operationally "tolerant" patient who had discontinued IS for more than 3 years. Sixty-six healthy subjects (HS) were studied as controls.
Results: Overall, the alloreactive T cell population was found to be significantly increased in the 73 kidney recipients (mean ± SE: 15.03 ± 1.04% of CD4+ CD25high T cells) compared to HS (5.93 ± 0.39%) ($p < 0.001$). In the 5 patients with CHR, this population was highly expanded (31.33 ± 4.16%), whereas it was comparable to HS in the 8 stable recipients receiving minimal IS (6.12 ± 0.86%), in 4 patients who had been switched to sirolimus (4.21 ± 0.53%) as well as in the unique "tolerant" recipient (4.69%). Intermediate levels (15.84 ± 0.93%) were found in the 55 recipients with stable graft function on standard CNI-based IS. Regulatory T cells, defined as CD4+ CD25high FoxP3+ CD127low, were found to be significantly reduced in all recipients except in those with minimal or no IS, and this reduction was particularly striking in recipients with CHR.
Conclusion: After kidney transplantation, an alloreactive T cell population was found to be significantly expanded and it correlates with the clinical status of the recipients. Interestingly, in stable patients with minimal (or no) IS as well as in patients on sirolimus, alloreactive T cells were comparable the healthy controls. Measuring circulating CD4+ CD25high CD45RO+ CD127high T cells may become a useful monitoring tool after transplantation.

P247

Prospective monitoring of a CD4+ CD25high CD45RO+ CD127high T-cell population after first kidney transplantation following thymoglobulin or basiliximab induction

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Background: Recent data have suggested that a population of CD4+ CD25high T cells, phenotypically characterized by the expression of CD45RO and CD127, is significantly expanded in stable liver and kidney transplant recipients and represents alloreactive T cells. Induction therapies may have an impact on this alloreactive T cell population. In this study, we prospectively analyzed CD4+ CD25high CD45RO+ CD127high T cells after induction with either thymoglobulin or basiliximab.
Patients and methods: A total of twenty-seven kidney transplant recipients were prospectively enrolled; 14 received thymoglobulin induction followed by a 4-day course of steroids with tacrolimus and mycophenolate mofetil («thymo group»), and 13 received basiliximab induction followed by standard triple immunosuppression (tacrolimus, mycophenolate mofetil and prednisone) («BSX group»). Phenotypical analysis by flow cytometry of the expression of CD25, CD45RO and CD127 on peripheral CD4+ T cells was performed at 0, 3 and 6 months after transplantation. Twenty-four healthy subjects (HS) were studied as controls.
Results: There were no differences in baseline characteristics between the groups; at 6 months, patient survival (100%), graft survival (100%), serum creatinine (thymo group versus BSX group: 129 versus 125 micromol/l) and acute rejection (2/14 versus 2/13) were not significantly different. Thymo induction produced a prolonged CD4 T cell depletion. As compared to pre-transplantation values, an expansion of the alloreactive T cell population was observed at 3 months in both thymo (mean: from 6.38% to 14.72%) and BSX (mean: from 8.01% to 18.42%) groups. At 6 months, the alloreactive T cell population remained significantly expanded in the thymo group (16.92 ± 2.87%) whereas it tended to decrease in the BSX group (10.22 ± 1.38%).
Conclusion: Overall, our results indicate that the expansion of alloreactive T cells occurs rapidly after transplantation in patients receiving either thymo or BSX induction. Whether differences at later timepoints or whether different IS regimens may modify this alloreactive population remains to be studied.

P248

Assessment of immune suppressive circuits in the tumour micro-environment of human head and neck squamous cell carcinoma (HNSCC)

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The presence of tumor infiltrating lymphocytes (TILs), notably CD8+ T cells, in several types of tumors, has been correlated with good prognosis. Moreover, some T cells among TILs have been shown to kill tumor cells in vitro through a tumor-associated antigen recognition mechanism. In contrast, accumulation of another subset of T cells, regulatory CD4 T cells (FoxP3+), may correlate with poor prognosis. In general, most tumors progress despite naturally acquired immune responses. Reasons for failure of adaptive anti-tumor immunity may be the usually weak immunogenicity of tumor cells and antigens, and a large variety of immunosuppressive mechanisms present in the tumor microenvironment.
Despite advances in the diagnosis and treatment of HN cancer, survival rates for this disease have not improved over recent years. New therapeutic strategies, including immunotherapy, are potentially useful approaches. In this regard, a better understanding of the cellular and molecular interactions taking place at tumor sites is required for the rational development of therapeutic interventions. Such insight would allow identifying novel immune modulators, which could be used to enhance vaccine specific immunity and increase both specific T cell responses and clinical response.
Our analysis of 46 HNSCC demonstrated that MAGE-A3/-A4 cancer testis antigens were expressed in over 70% of samples, indicating that these antigens are suitable targets for immunotherapy. Immunohistological studies showed that over 50% of tumors had a moderate to strong infiltration for CD3+, CD8+ and S-100+ cells. We further evaluated the distribution of BDCA-2+, CD11+, CD56+ and FOXP3+ cells in the same cohort of patients. The expression of IDO, iNOS, Arginase, Bcl-2 and Cox-2 was also evaluated by immuno-histochemistry, and assessed for correlation with clinical data. BDCA2+ and FOXP3+ cells were observed in all tumor samples, both in cancer stroma and within cancer epithelium. CD11+ and CD56+ cells were poorly present and no significant difference was observed between stroma and epithelium. Analysis of expression of IDO, iNOS, Arginase, Bcl-2 and Cox-2 are currently ongoing.
Together, our data reveal the presence of immune regulatory cells. The relatively easy accessibility of Head and Neck tumors provides an essential basis for further exploration, and a more precise characterization of immunosuppressive mechanisms revealed by ex vivo analysis of human cancer tissue.

P249

Single nucleotide polymorphisms in the distal promoter of ST2 in psoriasis and atopic dermatitis

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ST2 belongs to the IL-1R family and is a specific receptor for IL-33. It exists in two forms, as a long, membrane-bound receptor (ST2L) and as a short soluble protein (sST2), and is mainly expressed by basophils. Additionally, mast cells express ST2L, where it acts as a co-stimulation receptor for mast cell activation. There are two different promoters, the distal promoter, used by both forms, and the proximal promoter, used only by sST2. Two single nucleotide polymorphisms (SNPs) in the distal promoter of the ST2 gene at the positions -26999G/A and -27639A/G have been described to be associated with atopic dermatitis (AD) by altering the expression of both ST2 forms. As this study was performed with Japanese AD patients, we tried to confirm theses data with Caucasian AD patients. In a pilot study with 37 patients suffering from AD and, as a control group, 11 patients with psoriasis (Pso), we investigated these two polymorphisms.
No significant differences in allele frequencies were observed between the two groups. The frequency of the AD-susceptibility-allele ST2 -26999A was lower in the AD group (0.27) compared to the Pso group (0.41), whereas at the second locus, the AD-susceptibility-allele ST2 -27639G frequency was higher in the AD-group (0.51 in AD vs. 0.41 in Pso). Interestingly, the combined heterozygous genotypes at both loci (ST2 -26999GA and ST2 -27639AG) are significantly over-represented in the Pso group, while combined genotypes containing recombined haplotypes were over-represented in the AD-group ($p = 0.048$). The two loci are completely linked in the Pso group, whereas at least 13 out of 37 (35%) AD-patients carry recombined haplotypes. These data may either suggest that recombined haplotypes of the two SNPs in the distal promoter of ST2 may be a marker for the susceptibility for atopic dermatitis, or these SNPs may also play a role in the pathogenesis of psoriasis.

P250

High expression of indoleamine 2,3-dioxygenase gene as malignancy signature in prostate cancer

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Purpose: Prostate cancer (PCA) is a leading cause of cancer-related death in men. Immunosuppressive factors such as Arginase 2, inducible and endothelial Nitric-Oxyde Synthase (iNOS, eNOS) and IL-6 play a role in functional impairment of the immune system in PCA patients. Little is known about their comparative expression in PCA and benign prostatic hyperplasia (BPH). Furthermore, Indoleamine 2,3-dioxygenase (IDO) produced by tumor cells and eventually by infiltrating Dendritic Cells (DC), initiates the degradation of Tryptophan (Trp) along the kynurenine pathway resulting in the production of immunosuppressive Trp catabolites known to suppress T cell proliferation and apoptosis. IDO expression has thus been proposed as a possible mechanism facilitating induction of immune tolerance towards cancer, and has indeed been reported in a limited (n = 11) number of PCA cases, but no comparison with BPH or functional data were provided.
We evaluate the expression of genes encoding immunosuppressive factors such as IDO in PCA and BPH tissues.
Methods: Gene expression in PCA and BPH tissues were evaluated by quantitative RT-PCR. IDO protein expression was evaluated by immuno-histochemistry. Trp and its catabolites concentrations in patient sera were evaluated by HPLC.
Results: IL-23, IL-17, Arginase 2, iNOS and eNOS genes were not more expressed in PCA tissues as compared to BPH. IL-6 gene expression was significantly ($P = 0.00018$) enhanced while TGF-b gene expression was significantly ($P = 0.035$) decreased in PCA tissues as compared to BPH.
IDO gene expression was significantly ($P = 0.00001$) enhanced in PCA tissues as compared to BPH, and correlates with alpha-methylacyl-CoA racemase A (AMACR A) gene expression ($P = 0.004$). Immuno-histochemistry showed IDO in endothelial cells. However, in PCA tissues showing evidence of high specific gene expression, IDO was focally detectable in tumor cells. In patients bearing these tumors, IDO gene expression correlated with Kynurenine/Tryptophan ratio in sera.
Conclusions: These data indicate that high expression of IDO gene is only detectable in PCA tissues. This suggests that IDO expression might be of potential relevance in the suppression of tumour specific immune responses in PCA and consequently represent a malignancy signature in prostate cancer.

P251

Is PFAPA syndrome a sporadic disease?

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Background: PFAPA syndrome is a recurrent febrile disease first described in 1987 by Marshall and characterized by periodic fever, aphtous stomatitis, pharyngitis and cervical adenitis. Since the first description no clear etiology has been found.
In opposite to other auto-inflammatory diseases, no genetic origin was underlined and no familial tendency was reported until now. To better understand this disease, we created a European multicentric registry with the participation of 8 countries and 14 rheumatologic centers.
Aim: to investigate the eventual familial tendency to present PFAPA or an other chronic inflammatory disease.
Patients and methods: in 2 of the participating centers (Lausanne-Geneva, n = 45, and Bordeaux, n = 39), we questioned all patients or their parents during a phone call interview to complete the family history. We used the same questionnaire for a control group taken from a general pediatric consultation. They were asked for positive family history of recurrent fevers, PFAPA, and rheumatologic diseases (chronic inflammatory).
Results: Family history for recurrent fever was positive in 19/45 and 18/39 PFAPA patients from Lausanne-Genève and Bordeaux respectively, and always negative in the control group. 6/45 and 3/39 PFAPA patients had a family member with PFAPA, but none in the control group. The differences between both PFAPA groups and the controls are statistically significant. The family history for rheumatologic diseases (chronic inflammatory) was more frequently positive in the Swiss PFAPA group (14/45), than the French PFAPA group (5/39) and the controls (2/34).
Conclusion: These results suggest a familial susceptibility and a potential genetic origin for the PFAPA syndrome. This opens a wider spectrum for future research.

P252

Print of the frontal median vein: a differential diagnosis of the linear scleroderma "en coup de sabre". Interest of the scanner in 3 dimensions

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Background: The linear scleroderma "en coup de sabre" is a linear cephalic scleroderma, generally paramedian. Its diagnosis is clinical, sometimes difficult in the initial forms; histology is useful in uncertain cases but induces scars. We report two cases of linear mediofrontal depression corresponding to a print of the frontal median vein (FMV), of patients initially addressed with a diagnosis of a starting linear scleroderma. Diagnosis could be corrected by the clinical aspect and by the use of a scanner with reconstruction in 3 dimensions (CT3-D).
Methods: Case 1: a 35-year-old woman, treated for a frontal melasma (peeling), noted the persistence of a linear medio-frontal pigmentation with a cutaneous depression, without cutaneous atrophy or sclerosis.
A CT-3D did not show attack of bone or soft tissues, but a FMV particularly developed in vascular window, in regards of melasma.
Case 2: a 21-year-old woman, treated for a parietal alopecia areata by intralesional Kenacort ® with cutaneous atrophy post- injection, noted the appearance of a medio-frontal linear depression. The CT3-D did not show bony attack, only a discreet depression of soft tissues in regard to the FMV and one parietal cutaneous atrophy in the site of injection of Kenacort®. In both cases, diagnosis of linear scleroderma was rejected.
Results: The FMV is a vein which assures the venous draining of a part of the face. It seems to be particularly developed in certain patients, which can resembles of linear mediofrontal drainpipe. Concerning our patients, diagnosis of linear scleroderma was unlikely due to the absence of cutaneous sclerosis, and to mediofrontal and not paramedian topography; more so, reconstructions in 3-D of the scanner allowed to make coincide the path of this frontal vein with the linear depression. This depression was noted by our two patients further to a medical treatment close to the frontal zone. It probably attracted their attention to this region, explaining the haphazard discovery of this anatomic variant.
Moreover, a case of mediofrontal melasma miming a linear scleroderma was reported, the diagnosis having been specified by a biopsy.
Conclusion: The print of the frontal median vein is to take into account in the differential diagnosis of the initial linear scleroderma "en coup de sabre".
In case of diagnostical doubt, a CT3-D with vascular window allows to specify this diagnosis without cutaneous biopsy in irreversible aesthetic consequences.

P253

Severe recurrent Still's disease requiring IL-1 receptor antagonist treatment in a 17 old woman

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Background: Classical treatment of Still disease – a rare heterogenous autoinflammatory disease – comprises anti-inflammatory drugs, corticosteroids and other immunosuppressives like methotrexate, but some severe cases fail to respond to these drugs and require thus alternative approaches.
History: This 17 old year student consulted during spring 2006 an ENT doctor for pharyngitis. She then developped feverish night sweats, severe pruritic skin eruptions and arthralgia (wrists, ankles, hips) with an episode of arthritis (dactylitis). She furthermore presented with a diffuse skin eruption (mixed of fixed type urticaria and maculopapular exanthema) with dactylitis. Laboratory tests demonstrated moderate inflammation (ESR 55 mm/1h, CRP 73 mg/ml, leukocytosis 11.5 G/L and thrombocytosis 430 G/L). All immunological markers were negative. Ferritin base level rose from 17 to 168 ng/ml when she had dactylitis. The skin biopsy revealed leucocytoclasic vasculitis. The diagnosis of Still disease was strongly suspected. In June 2006 she received an oral tapering corticosteroid course, hydroxychloroquine 2x200 mg/d, levocetirizine 5 mg and doxepin 50mg. Under this treatment the skin exenthema diminished partly, but diffuse joint pain persisted requiring thus methotrexate treatment (15 mg weekly injections) since October 2006. Visual disturbances appeared in December 2006 in relation to optical neuropathy. Hydroxychloroquine was stopped and visual disturbances fully improved. One year later in October 2007 diffuse joint pain and right wrist arthritis occurred, resistant to oral steroid treatment, requiring intraarticular corticosteroid infiltration. Because of relapse of joint pain (under Methotrexate and oral corticosteroids) and the reoccurrence of febrile accesses, it was decided in November 2007 to initiate IL-1Ra treatment (Kineret ® = 100 mg sc/d) which is currently well tolerated and successful.
Conclusion: This case of Still disease with its atypical severe skin presentation highlights the frequent severe recurrent course of this disease and supports – upon failure of combined conventional immunosuppressive therapy – the use of novel approaches such as biological treatment with anti-IL-1Ra.

P254

Tumour antigen-encoding MRNA for the analysis of spontaneous and vaccine-induced immune responses in cancer patients

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Introduction: For the development of effective cancer vaccines there is a requirement for the assessment of vaccine induced immunity. Current immunomonitoring strategies do not allow for the optimal investigation of the full breadth of T cell responses, and is hampered by the limited number of known epitopes for most tumour antigens.
Methods: In this study transfection of antigen-presenting cells (APC) with modified mRNA constructs encoding for tumour antigens was optimized. mRNA encoding for full length NY-ESO-1 and CT-7/MAGEC1 has been applied to monitor T cell responses in cancer patients with naturally occurring immune responses to their tumour or following vaccination.
Results: CD8 T cells obtained from lung cancer patients with humoral immune responses directed towards NY-ESO-1 could be successfully amplified in vitro following only one stimulation round with mRNA-transfected APC. Specific killing of a panel of HLA-matched allogeneic NY-ESO-1 expressing tumour cell lines by the monoclonal CD8 T cells indicates an oligoclonal response including a novel HLA-B49 restricted epitope. Detection of NY-ESO-1 specific CD4 T cells in patients could be enhanced using a modified mRNA construct that targets the MHC class II pathway. The establishment of functional CD4 T cell clones specific for NY-ESO-1 has enabled the definition of the restriction element HLA-DQB10301 and HLA-DPB10402. Oligoclonal CD8 and CD4 T cell responses were detected in patients following an NY-ESO-1 vaccination. Using a modified CT-7 encoding mRNA, CT-7 specific CD4 T cells were detected in melanoma patients.
Conclusion: This methodology allows for a more precise monitoring of responses to tumour antigens in a setting that addresses the breadth and magnitude of antigen-specific T cell responses, and that is not limited to a particular combination of known epitopes and HLA-restrictions.

P255

Nausea and vomiting as indicator of acute bronchospasm in a patient with ASS-intolerance without asthma

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Background: The incidence of aspirin hypersensitivity in the general population ranges from 0.6–2.5%, but increases in adult asthmatics up to 11%. Controlled challenge tests (inhalative or oral) with acetylsalicylic acid (ASS) or other nonsteroidal antiinflammatory drugs (NSAID) are the diagnostic tool of choice. Symptoms of ASS hypersensitivity can display a wide range of clinical pictures such as an acute bronchospasm, urticaria, angioedema, chronic rhinitis, myocardial ischemia and anaphylactic shock.
Case report: A 42-year old patient was admitted to our clinic for further investigation of chronic nasal obstruction. Twice sinus surgery was performed because of recurrent severe polpyosis nasi with no persistent success. There was no known asthma by history. He reported nausea, vomiting or urticaria after ingestion of ASS or NSAID. Neither blood eosinophilia nor elevated exhaled nitric oxid (FENO) were detected. Both, the challenge test with methacholine (>2.4 mg) as well as the inhalative L-ASS-provocation (> 60 mg) test were negative. Oral provocation with ASS was performed starting with 80 mg. At the cumulative dose of 240 mg ASS, a drop of the PEFR from 580 to 470 l/min was observed . However, the patient felt well and had no respiratory symptoms. After the cumulative dose of 400 mg ASS, the patient felt nausea without any respiratory distress. PEFR was 320 l/min, and a bradycardia of 54 bpm was noticed. After vomiting he realized some dyspnoe. Because an asthmatic reaction was obvious, inhalative salbutamol and adrenaline as well as systemic antihistamines and corticosteroids were administered.

Conclusion: Nausea eventually followed by vomiting can mascarade acute bronchospasm due to ASS intolerance. Because of concomittant bradycardia vagal stimulation may be the most likely explanation for this “gastro-bronchial” reaction.

P256

Contact sensitivity to metal in patients with sarcoidosis and patients with metal implant

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Introduction: Sarcoidosis is a chronic granulomatous disorder with a strong genetic background, mainly affecting the lung, skin and the eyes. Association to exogenous factors such as M. tuberculosis and Propioni bacteria as well as inorganic dusts, insecticides, moulds and pollens has been proposed. Beryllium has the potential to induce a pulmonary disease mimicking sarcoidosis, other metals related to granulomas are aluminium, zirconium and titanium. We evaluated the prevalence of positive patch tests to metals in patients with confirmed sarcoidosis compared to patients with putative symptoms from metal implants.
Patients and methods: Eighteen consecutive patients with sarcoidosis (8 females, mean age 48 years) and 10 patients with metal implants (5 females, 52 years) were included. All underwent an extensive history for occupational and other metal exposure, and were patch tested with the standard and a metal series, an extended metal series was used in 12 patients. Readings were done at day 2 and 3 according to the ICDRG, and patients were instructed to report and present with late reactions. In a subset of patients also LTT were performed.
Results: In the sarcoidosis group 6/18 (30%) were positive to metals: 4 to manganese, 2 to nickel, and one each to gold and titanium. In the metal implant group 7/10 (70%) were positive to metals: 5 to manganese, 3 to vanadium, 2 to rhodium and one each to nickel, cobalt, gold, titanium and niobium. In each group 1 patient developed a late reaction at day 5/6 to beryllium. Six in the sarcoidosis group, and 4 in the implant group reported occupational metal exposure. 44% (4 osteosynthesis, 4 dental) sarcoidosis and 70% (4 osteosynthesis, 3 dental) metal implant patients were metal implant carriers at the time of testing.
Discussion: We found an unexpected high prevalence of positive patch tests to metals in sarcoidosis and metal implant patients. There was a low prevalence to the classical metals nickel, cobalt and chromium. The majority of the positive reactions, however, could be related nor to exposure nor symptoms. Active sensitization may present a problem particularly with beryllium. In 2 individuals a late reaction occurring at the limit of the typical chronological pattern of active sensitization was observed. Metal contact sensitivity may play a contributing role in sarcoidosis and symptomatic metal implants.

P257

Allergic asthma to mealworm (Tenebrio molitor) in a caretaker of dwarf bats (Pipistrellus pipistrellus)

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Introduction: Allergic symptoms to furred pet animals are common, however, IgE mediated allergic reactions to bats have been rarely reported. Typically, the affected individuals had intense occupational contact with bats or the respective allergens.
A patient is reported, who developed severe rhinoconjunctivitis and asthma while caring for dwarf bats. IgE-mediated sensitization to the feed (Tenebrio molitor) and bat droppings was demonstrated. Upon proper treatment and prevention, the symptoms rapidly resolved.
Patient: A 38 year-old Swiss female patient has had allergic rhinoconjunctivitis from pollen since childhood, in the last years, symptoms became less prominent. Two years ago, she started to care for dwarf bats (Pipistrellus pipistrellus) and has particularly raised bats by feeding them mealworm (T. molitor). After approximately 1½ years, she started to develop severe rhinoconjuncitivits and asthma while feeding and handling the bats. The patient was referred for further allergologic work-up.
Methods and results: Atopy screening was positive for beech, plantain and grass pollen. An animal allergen series and a flour allergen series were both negative. Tests with bat hair, bat feed (T. molitor), bat droppings from T. molitor-fed bats, T. molitor feed (oat bran) bat droppings from insect-fed bats, rat hair, rat droppings, and rat feed were performed. Positive results were obtained with bat droppings from T.molitor fed bats, T. molitor and used oat bran to feed T. molitor.
Total IgE were 243.00 kU/l; SX1: 1.99 kU/l; epithelial mix ex70, cereal mix fx3 and moulds mix mx1, rat and mouse allergen mixes were all <0.10 kU/l; T. molitor: 32.00 kU/l (class 4); an externally performed RAST to bat hair and bat droppings was elevated at 7.40 kU/l and 7.91 kU/l, respectively.
1 bat-exposed individual and 2 non-exposed individuals were negative in all skin tests with implicated bat allergens.
Discussion: Our patient developed severe allergic symptoms while caring for dwarf bats. Initially, a sensitization to allergens from the bat itself was suspected. However, in skin tests and by specific IgE, a sensitization to the bat feed Tenebrio molitor could be demonstrated. The positive skin tests to bat droppings from bats fed with T. molitor implicate that some antigens of T. molitor might be resistant to digestion. This could explain symptoms while handling bats and contact with bat droppings. This issue is addressed by further analysis using RAST inhibition assays.

P258

Sensitisation to wasp venom by one accidental injection in a patient desensitised for bee venom allergy

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Introduction: In the diagnosis of hymenoptera venom allergy intradermal tests with venoms are routinely used. There is a low risk of anaphylaxis from skin tests, and an even more remote risk of active sensitization. A case of a 23-years-old Swiss woman is reported, who accidentally received one full therapeutic dose of wasp venom extract (Alutard) during VIT for bee venom allergy and who newly developed specific IgE.
Patient’s history: 3.5 years ago, bee venom SIT was started due to a grade III anaphylactic reaction from a bee sting. At that time intradermal tests and specific IgE were positive for bee venom (1.0 µg/ml, 0.47 kU/l) and negative for wasp venom (up to 0.1 ug/ml, <0.35 kU/l). After 3 years control measurements were still positive for bee and negative for wasp venom. No field stings had been experienced. 4 months later she accidentally received a maintenance dose of 100’000 SQU wasp venom (ALK) instead of the usual bee venom. The patient had a mild local reaction from the accidental injection. Specific IgE and IgG for both venoms were measured by CAP RAST FEIA one and 5 weeks and 6 months later.
Result: Specific IgE were positive to bee venom (0.47 kU/l) and negative for wasp venom (<0.35 kU/l) at the start and after 3 years of VIT (bee 0.58 kU/l; wasp <0.35 kU/l), whereas specific IgG were increasing to both venoms. 1 week after the accidental injection specific IgE were unchanged (bee 0.58kU/l; wasp <0.35mg/l), after 5 weeks she developed specific IgE to wasp venom (4.40 kU/l) and further elevation of prior existing specific IgG (from 2.70 mg/l up to 9.09 mg/l). Those values remained elevated 6 months later (IgE 2.73 kU/l, IgG 6.16 mg/l).
Discussion: Our case shows that one single subcutaneous injection of wasp venom extract can induce longlasting specific IgE and booster IgG production. The increased production was demonstrated up to 6 months after the injection. Due to ethical considerations intradermal skin tests were not done to prevent boostering. The clinical relevance of the sensitization remains unclear since a field sting or sting provocation test has not taken place.

P259

Delayed hypersensitivity to antibiotics – value of diagnostic methods

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Introduction: Antibiotics are among the most frequent elicitors of T-cell mediated allergic reactions to drugs. Sensitivity and specificity of the available diagnostic means are for some antibiotics not very high and heavily dependent on the experience of the investigator, the performance of the tests, the accuracy of the history and clinical diagnosis. We evaluated the diagnostic value of skin tests and cellular assays for establishing the diagnosis in antibiotics induced type-IV allergic reactions in relation to the clinical diagnosis as established by detailed history and clinical symptoms.
Methods: All charts of patients who presented with a type-IV allergic reaction following intake of any antibiotic during a 3 year period were evaluated retrospectively. The putative causative agent at the beginning of the examination was compared to the final diagnosis and the diagnostic means (skin prick test, intradermal test (IDT), patch test (PT), lymphocyte transformation test (LTT) leading to the

diagnosis was established. Final diagnoses of cases with negative test were also compared to the initial diagnosis.
Results: On average, 35 patients (mean age 38.2 years) were investigated for type-IV allergic reactions induced by antibiotics per year. 80% demonstrated a maculopapular exanthema, 6% each SDRIFE or DRESS and 8% miscellaneous reactions. The average delay between reaction and examination was 2.4 years. The most common elicitors were penicillins/aminopenicillins (71%), followed by cephalosporins (10%). In only 37%, a sensitization could be demonstrated (25% positive in IDT, 10% in PT, 22% positive in LTT).
Discussion: This study retrospectively investigates the reliability, positive and negative predictive value of diagnostic tests in the diagnosis of type IV allergic reactions to antibiotics and therefore gives information on the value of the diagnostic tests in different situations.

P260

Absence of clinical cross-reactivity between ragweed and mugwort pollen in co-sensitised patients

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Background: Ragweed pollen (Ambrosia artemisiifolia) is a potent allergen with a pollen peak in central Europe in the late summer months. In the incidence of allergic disease caused by the pollen of mugwort (Artemisia vulgaris) is between 10 and 14% among pollinosis patients. Several reports demonstrated that ragweed and mugwort share common allergens which result in serological and skin test cross reactivity of so far unknown clinical relevance. Clinical cross-reactivity could have a major impact on public health issues.
Methods: Among 787 consecutive patients during one year we found 64 patients (8.1%) sensitized to ragweed and/or mugwort. From those, 24 (14 females, median age 41.6 years), participated in the study. 10 non atopic persons served as control. Skin prick tests with ragweed and mugwort pollens were performed in duplicate with extracts from two different providers (Allergopharma 50000 BE/ml, ALK Abello 10 HEP). Total IgE and specific IgE (sx1, ragweed, mugwort (Phadia)) were measured. Conjunctival provocation tests with mugwort (Allergopharma 106:5000 SBE/ml) and ragweed extracts (ALK 302:10BU/ml) were conducted to evaluate the clinical relevance of the sensitization to the two pollen.
Results: 20 patients (83.3%) were sensitized to both ragweed and mugwort in the prick test, 4 (16.6%) to ragweed only. The skin prick test with the two ragweed extracts was congruent in 19 patients (79.2%). Among those 24 patients 3 (12.4%) had a negative atopy screen and were positive to Asteraceae pollens only. Specific IgE to ragweed were positive in 16 (66.6%), to mugwort in 14 (58.3%) patients. 2 patients (8.33%) reported symptoms during late summer, 18 patients (75%) in spring and/or early summer only, whereas 4 patients (16.6%) were asymptomatic. In 9 patients conjunctival provocation tests with mugwort were positive, whereas none reacted to ragweed. None of the control group was positive in any test.
Conclusion: In the area of Basel exposure to ragweed pollen is currently low, however, sensitization to ragweed pollen has been shown to become more prevalent. A large number of patients sensitized to mugwort pollen appears to crossreact to ragweed, in our study we found a high number of co-sensitized patients. However, no positive provocation to ragweed was observed, also among the 9 patients positive to provocation with mugwort. This indicates that on a clinical level cross-reactivity between mugwort and ragweed pollen is currently unlikely.

P261

Efficacy of immunotherapy in children with IgE-mediated anaphylaxis to hymenoptera venom: how many are protected at re-stings?

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Background: Hymenoptera venom allergies in children are of great concern because young patients tend to be more prone to re-stings than adults.
Objective: This study aims to determine the protective effect of venom immunotherapy (VIT) with and without use of emergency rescue medication (ERM) to re-stings in childhood.
Methods: 83 children (mean age 9.2 years; range 3.7–15.5) 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 between June 2006 and June 2007. Mean follow-up period was 7.7 years (range: 0.3–14.3 years) after commencement of VIT and mean duration of venom therapy was 3.6 years, with 50% of patients still undergoing VIT at time of survey. Primary endpoints included number of re-stings and percentage of systemic reactions to re-stings. As secondary endpoint we evaluated how many patients were carrying and taking ERM at re-sting. Furthermore, we investigated clinical efficacy of ERM in addition to VIT.
Results: 49 children (59%) had been re-stung 108 times by the insect they are allergic to. The rate of re-stings was 0.23 per patient per year of follow-up with no difference between bee and wasp stings. However, there was a trend to more frequent systemic reactions in children re-stung by bees (16%) as compared to wasp re-stings (6%, p = 0.25). The majority of patients (87%) carried ERM, but only 75% were taking it after re-sting. None used the adrenaline auto-injector. Children not using ERM experienced significantly more frequent systemic allergic reactions to re-stings compared to those taking ERM (19% versus 4%, p <0,01). Furthermore, grades of systemic reactions were milder in children taking ERM after re-sting with 3 grade I reactions in comparison to the reactions in children not taking ERM after being re-stung: 2 grade III reactions with need of emergency medical attention, 1 grade II, and 2 grade I reactions.
Conclusions: A majority of children are being re-stung after commencement of VIT with no difference in prevalence of bee and wasp re-stings. VIT in children proves to be a successful therapy providing complete protection in 94% of children with allergy to wasp-venom and in 84% with allergy to bee-venom. ERM (oral antihistamines and steroids) provides an effective adjunct therapy with reduced incidence and severity of systemic allergic reactions to re-stings.

P262

Anaphylactic shock after intradermal testing with beta-lactam antibiotics

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Case: A 54 year old woman with preexisting asthma (treatment with ICS/LABA) and rhinopathy received oral amoxicillin/clavulanic acid (875/125mg) for a cutaneous infection, duration 1 week, treatment well tolerated. 1 week after cessation an exanthema developed, which was assessed by a dermatologist as morbiliforme like, accompanied by rhinoconjunctivitis and dyspnoea. Symptoms lasted for 2 weeks. No other medication.
Advanced history: 10 years ago generalized urticaria and bronchoconstriction within 60 minutes after an oral multivitamine preparation, which was once reproduced by the pa-tient herself. No intolerances to any drugs or foods are known, and she never had skin symptoms.
Allergological work up 4 weeks later: Relatively high basal serum tryptase (11,0 ug/l*), elevated total serum IgE (298 kU/l*), slightly elevated Penicilloyl G specific serum IgE (0,51 kU/l*), and normal IgE to Penicilloyl V and Amoxilloyl (<0,35 kU/l*). Intradermal test were performed on the back with Histamine 1 :10.0000, NaCl 0,9 %, PPL** 1:10 + 1:100, MDM** 1:10 + 1:100, Penicillin 10.000 IU/ml, Amoxicillin 25 mg/ml + 5 mg/ml, scratch test with clavulanic acid 10%, all under latex-free conditions. (* Phadia Immuno-Cap, ** Diater TM.)
5 minutes after intradermal testing the patient developed anaphylaxis with abdominal pain, generalized erythrodermia, severe bronchospasm and cardiovascular collaps with amnesia (RR was not measured initially). Tryptase 2,5 hours after beginning of symp-toms was 25,6 ug/l. Due to emergency treatment the skin tests could not be evaluated.
Conclusion: Urticaria and rarely anaphylaxis are known to occur after i.d. tests, in particular with drugs. This case is unusual, as the clinical course was actually not very suggestive of a severe IgE mediated reaction, because clinical symptoms appeared 1 week after stop of treatment. Only the appearance of conjunctivitis and the flare up of asthma symptoms are retrospectively suspicious of an IgE-mediated reaction to amoxicillin/
clavulanic acid. Nevertheless, the acute clinical symptoms, the elevation of tryptase following skin testing and the moderately elevated specific IgE to penicilloyl indicate that the anaphylaxis after skin testing was IgE/mast cell related.
This case illustrate that i.d. tests should be performed cautiously and under readiness to treat anaphylaxis even when not expected.

P263

Postmortal detection of systemic mastocytosis in a patient with fatal Hymenoptera sting reaction without venom specific serum IgE antibodies

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Introduction: Mastocytosis and elevated baseline serum tryptase are associated with especially severe allergic reactions to Hymenoptera stings.
Case report: A 58-years old gardener was stung in August 2006 in his left ear during work, probably by a vespid. He rejected medical treatment proposed by his boss. About 45 minutes later he was found unconscious and pulseless. Resuscitation was not successful.
Legal inspection showed a punctate skin lesion without stinger on the left ear due to the Hymenoptera sting. Besides there was abundant, white foaming and partly bloody secretion in the oral cavity and the tongue was somewhat swollen.
At autopsy beside secretion mucosal petechiae were found in the oral cavity and in the stomach. Coronary arterioscleoris with subtotal stenosis was present in 3 main coronary arteries, and several old ischemic lesions in a papillary muscle and the ventricular septum as well as a more recent ischemic lesion in the posterior myocardium were reported.
Histologic examination of liver, kidney and bone marrow revealed systemic mastocytosis. In postmortal blood the serum level of tryptase was 1840 ng/ml (normal 0–11.4ng/ml). Total IgE was in the low normal range (18 kU/l) and no specific IgE to venoms of common European Hymenoptera (honey bee, Vespula, Vespa crabro, Dolichovespula and Polistes) were found.
A history of arterial hypertension, coronary heart disease and a transient ischemic cerebrovascular attack had been known for several years and the patient was under treatment with Coreniten®, Dilatrend® und Aspirin®cardio when stung. According to his family he had developed a reaction once to a bee sting but was never examined or treated for this.
Conclusions: This is the fourth reported case of a fatal Hymenoptera sting reaction in a patient with systemic mastocytosis. In contrast to the 3 other cases no sensitization to the venoms of European Hymenoptera could be found, suggesting that fatal Hymenoptera sting reactions in patients with systemic mastocytosis may occur without venom sensitization. Besides mastocytosis preexisting coronary and cerebrovascular arteriosclerosis and treatment with a betablocker and an ACE-inhibitor may have favoured the fatal course.

P264

How much IgE is specific?

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Background: Some clinicians still measure total IgE levels in atopic patients, others only that of specific IgE. The question remains nevertheless how much of the total IgE is actually specific. An approach was chosen to cumulate laboratory data from specific IgE determinations using almost 100 of the most tested allergens and determine their share of total IgE.
Methods and results: A database containing almost 100'000 specific IgE determinations was obtained from Phadia using CAP and ImmunoCAP (former UniCAP) systems. Tests were performed in the years 1988 to 2006 in 17 different countries. Sera had to be tested against at least 10 different allergens and yield at least one positive specific IgE test result in order to be included in our data bank.
In about 18% of sera, more than 90 allergens were tested. There was a tendency that in sera with a lower total IgE level also fewer tests were performed. Due to this bias we addressed the question whether using fewer allergens to calculate accumulated IgE would also result in a percentage-wise decrease of positive reactions for sera with a lower total IgE. Interestingly, the observed linear increase in the number of positive allergen extract tests in sera with increasing total IgE levels surpassed the possible effect of having tested a greater number of allergens. This indicates that donors with higher IgE levels also tend to recognize a greater number of allergens.
Another question was how much specific IgE can be measured using the panel of allergens used in our data bank. We obtained a triphased curve with 80% of the donors total IgE falling in the range of 50 – 1600 kU/l where a plateau was observed at about 40% specific IgE of total IgE. Furthermore, sera with low total IgE averages to approximately 60% specific IgE while high IgE sera – even though there is a greater number of allergens – show only levels of approximately 10% specific IgE.
Conclusions: As it is known, accumulated specific IgE and total IgE correlate poorly. However, we found that higher IgE levels also indicated a broader sensitization. This is the first time that in such a large database the percentage of specific IgE could be assessed. The question to be answered in the future concerns the specificity of IgE that can not be captured using our broad allergen set.

P265

Cross-reactivity between sulfasalazine and sulfamethoxazole

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Due to the wide use of sulfonamide drugs and rather common occurrence of hypersensitivity reactions, cross-reactivity of immune reactions to sulfonamides is of great interest. Sulfonamide drugs can be divided into 2 groups: aromatic amines (sulfonamide antimicrobials) and non-aromatic amines (diuretics, anti-inflammatory drugs). It is believed that there is no cross-reactivity between aromatic sulfonamides and non-aromatic sulfonamides. As all antimicrobials are aromatic amines, one tends to classify the crossreactivity of sulfonamides into those with or without antimicrobial function.
We analyzed the crossreactivity between the antimicrobial sulfamethoxazole and the anti-inflammatory acting sulfasalazine, which is a prodrug split by the bacterial enzyme azoreductase into 5-aminosalicylic acid and the aromatic sulfonamide sulfapyridine. Cross-reactivity was analysed using in-vitro lymphocyte transformation tests (LTT).
PBMC from 2 patients with severe hypersensitivity syndrome to sulfasalazine, 3 patients with sulfmethoxazole allergy and 5 healthy donors were isolated and incubated with medium only (negative control), two concentrations (10, 100 ug/ml) of sulfapyridine, two concentrations (100, 200 ug/ml) of sulfamethoxazole and tetanus toxoid (10 ug/ml) as a positive control. After 6 days of culture 3H-thymidine was added and cell proliferation was measured.
In all patients tested, the LTTs were positive to both sulfonamide drugs, namely to sulfapyridine and to the sulfamethoxazole, suggesting a strong crossreactivity to these drugs. None of the healthy donors reacted to any of the drugs tested. We abstained from provoking our patients with either sulfasalazine or sulfamethoxazole, as they had a clear and typical history, had rather severe symptoms and positive in vitro tests to both compounds.
The crossreactivity between the antimicrobial sulfamethoxazole and the anti-inflammatory compound sulfasalazine, respectively its metabolite sulfapyridine, is not obvious for most clinicians, as they are used for different purposes, and their structural relationship (both aromatic sulfonamides) is hidden and related to a metabolite. Therefore, patients with hypersensitivity to sulfasalazine or sulfamethoxazole should be specifically advised to avoid sulfamethoxazole or sulfasalazine, respectively. This is the more important as the respective T cell mediated reactions are life threatening and can cause severe generalized symptoms affecting liver, lung or pancreas.

P266

Iodine is not the allergen in delayed hypersensitivity reactions to contrast media

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Introduction: Hypersensitivity reactions to iodinated contrast media (ICM) are either immediate reactions (IT) occurring typically within one hour, or delayed reactions (DT), which typically occur after several hours to one or 2 days. In IT reactions, the pathomechanism is not clear, contradictory results with a very low up to a 70% prevalence of positive IDT have been reported. In DT reactions, delayed positive patch and IDT tests, and ICM-specific T-cells suggest a T cell-mediated mechanism. In both the role of iodine has not been clarified so far. Since occasionally positive skin tests to iodine containing drugs are observed, such patients are often falsely labelled as being "allergic to iodine". We investigated the presence of a hypersensitivity to iodine in patients with a history of hypersensitivity reactions to ICM.
Methods: 13 patients (5 male, 8 female, mean age: 61.6 years) with a history of IT-(group A, n = 5) or DT- (group B, n = 8) hypersensitivity reactions to ICM/iodine were investigated. Depending on the clinical reaction, skin prick tests, IDT and patch tests (PT) with several ICM

and different iodine formulations were done. After exclusion of contraindications and obtaining informed consent, all underwent oral provocation with pure iodine solutions.
Results: In group A, positive skin tests to 2 ICM were observed. One patient with completely negative skin tests reacted twice to oral iodine with an urticarial exanthem. In group B, a T cell-mediated sensitization to one or more ICM was identified in the skin tests in 7/8. In 6/8 patients additional contact sensitizations to one or more iodine formulations was found, one patient had an isolated contact sensitization to povidone iodine. Oral provocation with iodine was negative in 8/8 patients. Three patients experienced a transient nausea and dizziness during oral provocation.
Discussion: We have previously demonstrated in a patient with iodine mumps that oral challenge with Lugol's solution is in principle a valid means to elicit a hypersensitivity reaction to iodine. In these 12 patients we were able to show that in the majority iodine is not the eliciting agent in hypersensitivity reactions to ICM. Therefore, more likely the ICM molecules and not iodine are the eliciting structures. The only patient with an IT reaction to an ICM application and an urticaria to iodine did not have a detectable sensitization to a ICM.

P267

Chronic urticaria: cognitive flexibility influences the cause of urticaria

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A consultation for urticaria patients has been established at the Department of the University Hospital of Zurich since January 2007. Correlation between patient's history and type/cause of urticaria, impact of a positive autologous serum skin test (ASST) and of psychological factors on the course of urticaria were investigated. 98 patients with a mean age of 39 years (range: 11–77 years) and chronic or acute intermittent urticaria were recruited from January till December 2007. Patients were interviewed on suspected trigger factors of their urticaria and their cognitive flexibility was assessed using a Pictorial Representation of Illness and Self Measure (PRISM). ASST and physical tests (for pressure, cold, etc.) were performed. Patient's history correlated in fourteen patients with the identified type of urticaria (14,3%). Food allergy was suspected to cause urticaria by 32 patients (32,7%), physical triggers by 59 patients (60%), but could be only confirmed in five and four patients, respectively. The ASST was positive in thirty-five cases (54%). 65 patients (66,3%) demonstrated in PRISM analysis to have a cognitive flexibility, i.e. to be able to see a variation in the intensity and frequency of their symptoms. Thirty-nine of those patients (59,9%) showed a favourable course, i.e. a reduction of days with urticaria symptoms of 25%. Fifteen patients had no cognitive flexibility and only five of those (33,3%) showed a favourable course. However, there was no correlation between the outcome of ASST and the course of the urticaria. In conclusion: patient's history did not correlate with the clinical test results. More importantly, however, there is an indication that cognitive flexibility could be a predictive marker for the course of chronic urticaria.

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Qb-Feld 1, a potent and safe vaccine to treat cat allergy

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We generated a vaccine, consisting of the major cat allergen Feld1 coupled to bacteriophage Qb virus like particles (Qb-Feld1). To evaluate the efficacy of Qb-Feld1, we investigated IgE-mediated allergic reactions in vivo. Balb/c mice were first sensitized by intra peritoneal injection of Feld1 in the presence of Alum and immunized once either with Qb or Qb-Feld1. As expected Qb vaccination did not alter the preexisting antigen-specific humoral immune response. In contrast, vaccination of mice with Qb-Feld1 boosted anti-Feld1 IgG antibody titer without affecting the antigen-specific IgE immune response. Intradermal antigen challenge two weeks after vaccination induced a strong mast cell degranulation in the skin of Qb control but not of Qb-Feld1-vaccinated mice. Thus, treatment with Qb-Feld1 efficiently inhibited local immediate type allergic reactions. Serum transfer experiments revealed that this could be attributed to the allergen specific IgGs induced by Qb-Feld1 treatment. Next, the anaphylactic potential of Qb-Feld1 and free Feld1 was compared in vivo. Both, intravenous and subcutaneous administration of free Feld1 in sensitized mice induced a dramatic drop in temperature as well as vasodilation, two hallmarks of anaphylactic reactions. In contrast, Feld1 coupled to VLPs was devoid of anaphylactic potential with both routes of administration. Furthermore, Qb-Feld1 vaccination efficiently prevented systemic anaphylaxis upon subsequent intravenous Feld1 challenge. The strongly reduced toxicity of Feld1 displayed on VLPs could also be demonstrated with blood samples from cat allergic humans in vitro. In fact, Qb-Feld1 showed a roughly 100 fold lower propensity to induce degranulation of Basophils from cat allergic volunteers than free Feld1. Taken together the presented result shows that coupling of Feld1 to VLPs dramatically reduces its anaphylactic potential without affecting its immunogenicity. Hence, Qb-Feld1 vaccination may represent a safe and effective treatment for cat allergies in humans.

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Sensitisation to ragweed and mugwort in the Swiss SAPALDIA cohort

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Introduction: Ragweed (Ambrosia artemisiifolia) is increasingly found also in Switzerland, particularly in the area of Geneva and Ticino. Ragweed pollen has a potent sensitizing capacity and elicits allergic symptoms at very low levels. An increase of ragweed pollen has been observed since 1993. In Europe sensitization prevalence to ragweed pollen varies from 30% to 80% in allergic persons. Cross-sensitivity to other pollen from the Compositae family, such as mugwort (Artemisia vulgaris) pollen, is possible. Therefore, it is important to evaluate the prevalence of sensitization to both pollens.
Material and methods: In the SAPALDIA cohort study, the sensitization of the Swiss population to different common respiratory allergens was evaluated by Phadiatop® in the years 1991 and again in 2002. Sensitization to ragweed and mugwort was assessed in the blood sera collected in Aarau, Basel, Geneva, Lugano, Payerne and Wald in 2002 and at the same time in the blood sera collected in Geneva and Lugano in 1991. Specific IgE were determined by Immunocap (Phadia, Uppsala, Sweden) in all samples in 1991 and 2002.
Results: Complete data from both time points 1991 and 2002, was available from 4672 individuals. Positive in Phadiatop® were 28.8% and 30.1% respectively. In 2002, 322 (6.9%) had specific IgE to ragweed. Among the 1081 subjects from Lugano and Geneva, 101 (9.3%) were ragweed positive in 1991, as compared to 72 (6.7%) in 2002. Between the two surveys, 43 lost their sensitization to ragweed whereas 14 newly acquired it. Except for one subject (in 1991) all ragweed-positive subjects were either positive to mugwort and/or Phadiatop, and 81% of them were positive to mugwort.
Conclusions: Currently, the sensitization to ragweed and mugwort is within the range of other respiratory allergens. No significant increase of sensitization has been observed in the two locations in Switzerland where an increase of pollen has been reported. Isolated sensitization to ragweed is rare and in our longitudinal study no relevant new sensitizations to this potent allergen have been observed.

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Sulfanilamide compounds as agonists, partial agonists and antagonists of T-cell receptor activation

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Introduction: Many drugs evoke hypersensitivity reactions that are T cell-mediated, as evidenced by the generation of drug-specific T cell clones (TCC). Even though drugs are generally thought to act as T cell haptens, recent evidence suggests that different drugs – among them sulfanilamides like sulfamethoxazole (SMX) – act in a non-covalent way, presumably by reversibly interacting with either the major histocompatibility complex (MHC) or the T cell receptor (TCR).
Methods: To elucidate the molecular mechanisms of how sulfanilamides act on their specific TCR, a SMX–specific TCR was transfected into a TCR-deficient mouse T-cell hybridoma. Together with Ebstein Barr virus (EBV)-transformed B cells as antigen-presenting cells (APC), TCR downregulation as an early and partially agonistic, IL-2 production as a late and fully agonistic, and inhibition of IL-2 production as an antagonistic response were measured. SMX and 10 related sulfanilamides were used to pharmacologically characterize this particular TCR. Additionally, the parental T cell clone (TCC) and a T cell line (TCL) generated from SMX-expanded, peripheral blood mononuclear cells of the same donor were

characterized similarly and compared to the TCR transfectant.
Results: Almost all compounds were partial agonists as they led to TCR internalization on both the transfectant and the TCC. Generally, TCR internalization of the transfectant was more efficient than of the TCC. Only SMX induced a strong IL-2 secretion by the transfectant, while several related compounds led to IL-2 secretion and proliferation by the TCC (full agonists). This discrepancy was explained by dose response studies indicating that the TCC generally reacted much more potently than the transfectant. Hence, certain derivatives were able to activate the TCC but inhibited the transfectant, while others inhibited IL-2 secretion by both the TCC and the transfectant. Consequently, weak agonism on the TCC manifested itself as antagonism on the TCR transfectant in certain cases. TCR internalization, IL-2 secretion and inhibition thereof were also seen with the polyclonal T cell line.
Conclusions: In analogy to earlier results with altered peptide ligands, drugs can act as strong and weak agonists, partial agonists and antagonists of TCR activation, depending on drug concentration and cellular background. Providing easier handling than TCC, TCR transfectants will serve as a tool to start unravel the mechanism of drug-induced TCR activation.

P271

T-cell mediated responses to human and Aspergillus manganese superoxide dismutase(s) in adult patients with atopic dermatitis

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AD represents a chronic relapsing skin manifestation of atopy. It is well established that T cell responses to exogenous allergens represent a prominent feature of AD. Recently crossreactivity between fungal and human MnSOD (manganese superoxide dismutase) has been shown to be involved in the exacerbation of eczema in AD (Schmid-Grendelmeier et al. 2005, J Allergy Clin Immunol. 115:1068–1075). In this study we investigated the T-cell responses to the crossreacting human and aspergillus manganese superoxide dismutase(s) in more detail. Thirty patients (18 patients sensitized via IgE to Aspergillus fumigatus and 12 patients not sensitized to Aspergillus fumigatus) were included in our study. Both rh MnSOD (recombinant human manganese superoxide dismutase) and rhAsp MnSOD (recombinant Aspergillus manganese superoxide dismutase) induced proliferation of circulating T-cells labelled with CFSE and detected by flow cytometry. There was no significantly higher lymphocytic proliferation in AD patients compared to healthy individuals when stimulated with rh MnSOD and rAsp MnSOD .However in AD patients sensitized to Aspergillus fumigatus, rh MnSOD stimulated proliferation preferentially of the CCR4+(compared to CCR4-) and of CLA+(compared to CCR4-) putative skin homing T cells from the circulation. In contrast, in non sensitized patients there was no preferential proliferation in the CLA+ lymphocytes. MnSOD reactive T-cell clones (TCC) had been generated from the peripheral blood of AD patients. Interestingly more than 50% of these TCC were CD8+. In conclusion, our data provides preliminary insights into the mechanism(s) of autoallergic T cell responses to human and aspergillus MnSOD.

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Body mass index and atopic disease

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Background: Obesity and atopy are two increasingly important population health issues. Over weight and obesity are considered as potential risk factors for atopy, mainly asthma.
Aim: To determine body mass index (BMI) in children with atopic disease compared to non atopic children; to determine whether obesity is associated with increased severity of disease.
Methods: standardized questionnaire and clinical evaluation. The study involved children aged 5 to 17 years with diagnosed atopic disease that attended the Asthma School program. Children aged 5 to 16 years without atopic disease were used as a control group.
Results: A total of 202 children were included in the study: 102 children with atopy (70.1% asthma, 26.5% rhinitis and 3.4% eczema) and 100 children without atopy. The median age was 10.5 and 10.3 years for the atopic and non-atopic children, respectively. The prevalence of overweight or obese subjects was significantly greater in the atopic group (51.3% vs 41.1%, p 0.05). The obese asthmatic children had a significantly higher prevalence of sleep disturbances due to wheezing in the last 12 months (53.8% vs 21,6%, p 0.05). They also reported dry cough at night more often (56% vs 32,4%, p = 0.07), and a higher number of wheezing attacks in the past 12 months (72.7% vs 50.8%, p = 0.074), than the non-obese asthmatic children. Most of the obese atopic children were already overweight or obese at the time of the diagnosis (69,4%). No difference was detected regarding the gender.
Discussion: There is some evidence of an association between excess body weight or obesity and atopy-particularly asthma.

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Body mass index and asthma severity

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Background: The association between obesity and asthma severity remains rather controversial.
Aim: To determine the association between asthma severity and the body mass index (BMI).
Methods: We included children aged 5 to 117 years that participated in our Asthma School Program in the past 4 years. A total of 202 patients were divided into the following BMI categories: 108 (53.46%) non-overweight (BMI 5th to 84th percentile), 78 (38.61%) overweight (BMI 85th to 94th percentile) and 16 (7.9%) obese (BMI >95th percentile). Asthma severity measures included parameters such as respiratory symptoms, reliver medication use, night symptoms, hospitalization rate and symptoms triggered by physical exercise. Models were adjusted for: gender, age, parent smoking habits, family history of asthma.
Results: Compared with non-overweight subjects, overweight patients with asthma were more likely to report continuous symptoms (76% vs. 34%), use short acting beta agonists (68% vs. 28%), night symptoms (58% vs. 14%), hospitalization rate (2 hospitalizations pre year vs. 1 hospitalization pear year) and asthma symptoms triggered by physical exercise(38% vs.15%).
Conclusions: Over weight is associated with parameters of asthma severity.

P274

Anaphylactic shock due to chlorhexidine in a 62 years old patient during urologic surgery

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Background: Perioperative anaphylaxis during anaesthesia is mainly due to neuromuscular blocking agents, antibiotica and latex, but occasionally other compounds like dyes or antidesinfectants like chlrohexidine must be considered.
History: A 62 years old patient underwent prostate surgery (local spinal anesthesia) in January 2007. He presented during co-trimoxazole (Bactrim ®) infusion an anaphylactic shock (diffuse angioedema, dyspnoea with wheezing, hypotension and tachycardia). After shock treatment (volume replacement, i.v. adrenaline together with corticosteroids and anti-histamines) the intervention could be completed with general anesthesia. During subsequent urologic procedures urticaria and dyspnea occurred. History revealed an atopic disposition (pollinosis with asthma during childhood) without current allergic symptoms and no previous drug allergy. Operative history revealed no prior i.v. anesthetic drug administration, the use of intraspinal ropivacaine (Naropin®) and the urethral application of urethral gels during urologic surgery (Endosmed®) and subsequent urologic procedure (Instillagel®) containing both chlorhexidine and other components.
Allergic workup revealed positive skin tests for both gels (Instillagel ® and Endosmed®) and chlorhexidine whereas skin tests performed with all other components of both gels remained negative. I.d. tests with ropivacaine and co-trimoxazole were also negative. Atopy was underlined by positive tests for ash-pollen and house dust mites (D.far and D.pter) whereas all other aeroallergens, particularly latex were negative. Serological analysis (Phadia®) demonstrated an elevated IgE level (675 kU/l) and specific IgE levels at 1.32 kU/l for D.pter and of 0.63 kU/l for latex (k82). Determination of Specific IgE (Phadia®) for chlorhexidine is under way. Tryptase (basal value) was 8.5 µg/l (N <11,4).

Conclusion: This case of anaphylactic shock provoked by chlorhexidine hypersensitivity during surgery illustrates that this IgE-mediated allergy - occurring mainly during urological and dental interventions, but affecting also patients treated locally on the skin (wounds) with chlorhexidine – must be considered by allergists when a workup for anaphylaxis during anesthesia is performed.

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

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Introduction: Most of the hypersensitivity reactions to drugs are mediated by T cells. Currently available tests to detect T cell sensitization to a drug are skin tests and in vitro lymphocyte transformation test (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 (Beeler A, et al, Allergy. 2008; 63: 181-8). In contrast to LTT, CD69 measurement gives results in 48h and not only after 7 days and does not involve radioactivity.
Aim: In a prospective study we aimed to compare both in vitro methods as tests for diagnosis of drug hypersensitivity. Here we present preliminary data of first 33 out of planned 100 patients.
Methods: PBMCs from well defined drug allergic patients were isolated and stimulated directly with either culture medium (negative control), two different drug concentrations or tetanus toxoid (TT) as positive control. CD69 expression was measured after 48h by flow cytometry on CD3+CD4+ and CD3+CD8+ cells and on the whole lymphocyte population. In the LTT, proliferation was measured after 7d by 3H-thymidine incorporation.
Results: With both test we could detect sensitization to TT in 25/32 cases. In 4 cases only CD69 upregulation but not LTT, and in 1 case, only LTT but not CD69 was positive for TT. In 20/33 patients we couldn't detect any sensitization to the drug tested in both tests. In the remaining 13 patients only 4 were positive in both assays to the culprit drug, in 2 only LTT was positive and in 7 only CD69 measurement was positive, but not LTT.
Conclusions: These preliminary data indicate that CD69 measurement may be more sensitive to detect T-cell mediated response than LTT, but true sensitivity and specificity of both test can be determined only after evaluating all clinical data (symptoms, history and skin test, re-exposure).

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Differential recognition of contrast media by T-cells

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Background: Two to three percent of patients exposed to intravenously injected contrast media (CM) develop delayed type hypersensitivity reactions. Positive patch test reactions, immunohistological investigations, and CM-specific proliferation of T cells in vitro suggest a pathogenetic role for T cells.
Aim and methods: In this study, we investigated how T cells recognize CM. To this end we generated CM-specific T cell clones (TCCs) from peripheral blood of two patients with well documented delayed type hypersensitivity reactions to iohexol and iomeprol, respectively. Proliferative responses of TCCs to different concentrations of drugs were analyzed in specificity assays. To investigate the mechanism of drug-presentation, glutaraldehyde-fixed, drug-prepulsed, HLA-DR-matched or -mismatched antigen presenting cells (APCs), and HLA blocking antibodies were used.
Results: The stimulation of the CM-specific TCCs by CM was dependent on APCs and was MHC restricted (HLA-DR or -DP for CD4+, and MHC-class I for a CD8+ TCC). Two mechanisms of CM stimulation of T cells could be identified: the CD8+ and some CD4+ TCCs reacted to the CM independent of drug uptake and processing by APC, as glutaraldehyde-fixed APCs could present the CM. Other CM specific CD4+ TCCs required life APCs, compatible with uptake and presentation of CM on MHC-class II molecules, akin to the T cell reaction to a protein antigen like tetanus toxoid. Three findings imply such an uptake-dependent pathway: a) glutaraldehyde fixation abrogated presentation; b) after pulsing of the APCs with CM, the drug could not be washed away; c) time-dependent pulsing experiments demonstrated that 10 – 20 hours are needed for an optimal presentation of CM by APCs. Since allogeneic, MHC-matched APCs could present CM after pulsing as well, this ability of CM uptake and processing is not unique for APC from patients with CM-induced drug hypersensitivity.
Conclusion: Data suggest that CM are stimulatory for T cells either by a direct binding to the MHC-T cell receptor complex, or by an uptake-dependent way. Notably, both mechanisms appear to occur in parallel in the same patients.

The numbers refer to the pages of this supplement.