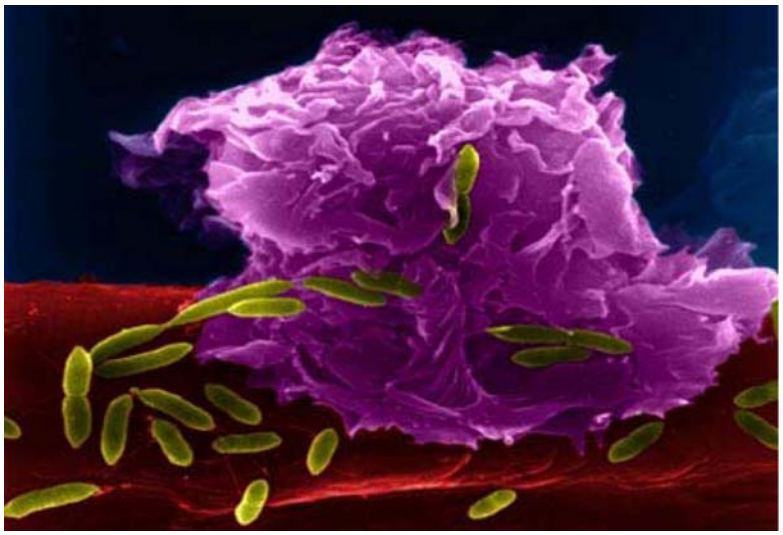
Comprehensive Center for Infection, Immunity and Transplantation

Infektiologie-Immunologie-Transplantation

# 1st CIIT SCIENCE DAY

July 1st, 2010

# Abstract book



tilak Universitätskliniken LKH Innsbruck

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# WELCOME

In 2009, the “Comprehensive Center for Infection, Immunity, and Transplantation (CIIT) (also visit: http://www.i-med.ac.at/innere_medizin/innere_medizin_1/CIIT) was established at the Medical University of Innsbruck. The CIIT is coordinated and organized by a speakers’ team from different disciplines and aims to promote and optimize the interdisciplinary collaboration and interactions in terms of clinical practice, science and teaching in these fields of interest at the Medical University of Innsbruck.

Therefore a series of lectures and case studies has been established to promote this interdisciplinary exchange. Within the CIIT colloquium local and external speakers present and discuss new interesting research topics in infection, immunity and transplantation. The Grand Rounds are clinically orientated and focus on the presentation and discussion of interesting clinical cases.

The 1st CIIT Science Day at the MUI will now bring together allocated researchers with a scientific focus in infection, immunity and transplantation and will thus provide an overview of current scientific topics and projects in the respective research areas. To this aim actual research projects or studies will be presented by the authors during guided poster tours. The organizers are very proud that almost 90 abstracts have been submitted which will guarantee a broad and fruitful scientific exchange.

In addition, we are very happy that Prof. Christian Bogdan, Head of the Institute for Microbiology and Immunology at the University of Erlangen, who is a well recognized European researcher in innate immunity and host-pathogen interaction, has agreed to give the keynote lecture at this 1st Science Day.

Finally, I would like to thank all the people who were involved in the organization of this 1st Science Day, specifically Andrea Schroll, Thomas Sonnweber, Patrizia Stoitzner, Susanne Perkhofer, Gerhard Blum, and the members of the CIIT speakers’ team as well as the poster moderators. We are also most grateful to our sponsors who provided significant financial support. They are acknowledged on the back of this abstracts book.

I wish all participants and guests a stimulating and interesting meeting and a fruitful scientific exchange.

Günter Weiss (CIIT- Speaker)

# Program

LOCATION: großer Hörsaal and Seminarräume MZA (Medizinzentrum Anichstraße)

Großer Hörsaal:

14.00-14.15 Introducing words: G. Weiss (CIIT-speaker)
Opening of the 1st CIIT DAY: Rektor Univ. Prof. Dr. H. Lochs

Seminarräume 1+2: Moderated Postersessions

14.15-15.15 Postersession I (Poster 1-28)

15.15-16.15 Postersession II (Poster 29-56)

16.15-17.15 Postersession III (Poster 57-85)

Großer Hörsaal

17.15-18.30 Keynote Lecture: Prof. Christian Bogdan, Univ. of Erlangen
Cutaneous and visceral leishmaniasis - from experimental mouse models to human therapy

18.30-20.00 Posterdiscussions with light buffet and drinks

| | | **Postermoderator** |
|---|---|---|
| 14.15-15.15 Postersession I | Poster 1-10 | Arthur Kaser |
| | Poster 11-19 | Matthias Schmuth |
| | Poster 20-28 | Lukas Huber |
| 15.15-16.15 Postersession II | Poster 29-37 | Arno Helmberg |
| | Poster 38-46 | Patrizia Stoitzner |
| | Poster 47-56 | Cornelia Lass-Flörl |
| 16.15 -17.15 Postersession III | Poster 57-65 | Nikolaus Romani |
| | Poster 66-75 | Gottfried Baier |
| | Poster 76-85 | Hubertus Haas |

Coffee will be available throughout the Postersessions.

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## 1. Human Platelets Alter Mitochondria of Aspergillus Fumigatus in vitro

S. Perkhofer[1*], L. Kremser[2], M. Nagl[1], B. Sartori[1], H. Lindner[2] and C. Lass Flörl[1]

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**Background:** The fungal pathogen Aspergillus fumigatus is responsible for an increasing number of fatal infections in immunocompromised hosts. Recently, we found that platelets exert antifungal effects against Aspergillus spp. by attenuating fungal germination and hyphal elongation, both of which are of major importance in evolving invasive disease. However, little is known about their target sites. Therefore we performed 2 D electrophoresis (2-DE), fungal mitochondrial function by examining the mitochondrial membrane potential and aconitase gene (acot) expression levels, an enzyme of the citric acid cycle.

**Methods:** In this study one clinical A. fumigatus isolate was used. After incubation of A. fumigatus hyphae with platelets at 37°C for 30 min, protein changes were analyzed by 2-DE and subjected to mass spectrometry. Also immunofluorescence analysis for detection of fungal m changes was performed by use of Mitotracker CMXRos (100mM). For expression level determination of fungal acot northern blot analysis and quantitative real-time RT-PCR was used.

**Results:** Incubation of platelets with A. fumigatus revealed a change of numerous proteins mainly associated to mitochondria as found by 2-DE. In support of these findings we observed that platelets induced a loss of mitochondrial membrane potential and down-regulation of fungal acot gene expression level in A. fumigatus.

**Conclusion:** Our results indicate that fungal mitochondria are targeted by human platelets and that the turnover rates of the entire citric acid cycle are massively diminished. Mitochondria are the energy machines and their impairment is associated with decreased cell function and cell death. The increased understanding on the role and target sites of platelets in innate immune defense against A. fumigatus is highly important to perform more specific and appropriate ways to combat this potentially devastating disease.

## 2. Molecular Epidemiology of Human and Animal Infections with Mycobacterium caprae

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**Background:** Mycobacterium caprae has been recognized as a pathogen causing tuberculosis in man and animals for a decade. In central Europe, approximately 1 % of human tuberculosis (TB) cases is caused by this pathogen. M. caprae has caused multiple outbreaks among wild-life and livestock in Western Austria during the last decade, constituting a threat to the surrounding normal population and, in particular, to immunocompromised persons.

In a multidisciplinary and multinational project together with epidemiologists and veterinarians, we apply genotyping to mycobacterial isolates to investigate potential transmission chains of M. caprae between species (animal-animal and animal-human). This, together with an estimate of prevalence, should allow in the end to propose measures to prevent/reduce transmission.

**Methods:** To characterize M. caprae isolates from animals or man, mycobacteria isolates are subjected to MIRU-VNTR typing in 25 loci (mycobacterial interspersed repetitive units - analysis of variable number tandem repeats), a DNA-fingerprinting method established as the gold-standard for molecular epidemiology or epizootiology of TB. Fingerprints are compared to a data-bank for M. caprae established in previous work.

**Results:** Since 2008, M. caprae from deer (n = 44), cattle (n = 21) and other animals (n=3) have been investigated. Thus, the prevalence of M. caprae infection among red deer in the afflicted four regions ranged from 20% to 0% (mean: 11%). All but 5 deer isolates showed complete identity to the outbreak prototype strain, with 5 isolates showing minor differences, i.e. in one locus or in two loci. In addition, all but one cattle isolate showed identical fingerprints. The single isolate clustered with outbreaks observed in Bavaria and tracing back animal trade in this case confirmed this origin. Comparison of the fingerprints with animal isolates from Germany and Northern Italy showed that this clone is multiply present, among others, in Bavaria and in one region in Val camonica (Brescia, Italy). Although exposure to M. caprae is documented for humans in several instances, no clinically manifest human TB case with M. caprae has been identified in afflicted areas both since 2008 and before.

**Conclusion:** We were able to quantify an unsuspectedly high prevalence of animal tuberculosis in certain areas in Western Austria. The data suggest the existence of a single stable and even more widespread M.caprae clone in Alpine wildlife. Nonetheless, transmission from animal hosts to man appears to occur very rarely. Further, multinational approaches are planned in order to understand and be able to control this zoonotic threat.

## 3. Dominance of CTX-M group 1 Enzymes in ESBL Producing E. coli from Outpatient Urines in Neighbouring Regions of North- and South Tyrol

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**Background:** Extended spectrum β-lactamases (ESBL), especially those of the CTX-M type are on the rise in Europe, not only in the hospital environment but also in outpatients. According to recent antibiotic resistance reports and data reported to EARSS there has been an impressive increase in 3rd generation cephalosporine resistance in *E.coli* from urine between 2002 and 2007 in North-Tyrol, by far exceeding the rest of Austria. Moreover the ESBL phenotype is associated with nearly 100% quinolone resistance in those isolates.

**Methods:** We performed a small comparative pilot study in ESBL producing *E.coli* isolated from outpatients suffering from urinary tract infections, from the Austrian North-Tyrol region, and from Bozen/South Tyrol (Italy). ESBL enzymes were typed with PCR and DNA sequencing and also plasmid mediated quinolone resistance genes were investigated.

**Results:** Using established PCR methods we detected in nearly 90% of ESBL producing *E. coli* isolates CTX-M group 1 enzymes and only a few group 2 or 9 enzymes, the latter only found in South Tyrol. *blaTEM, blaOXA-1* and aminoacyltransferase *aac(6')-Ib* were significantly more frequent in the Austrian North, where also blaSHV was found in only one isolate. The linkage of blaOXA-1 and aminoacyltransferase *aac(6')-Ib*, a facultative quinolone resistance marker, in the isolates from north Tyrol suggests that they are located on the same plasmid.

In 2009 the overall prevalence of ESBL in E.coli causing urinary tract infection in outpatients was 7% in samples of a local laboratory in Innsbruck and 5% in samples from the reference laboratory in Bozen, respectively. Only few plasmid mediated quinolone resistance genes were found, *qnrA* was found in an AmpC producing *E. coli* from Innsbruck and *qnrS* in two ESBL producers from Bozen.

**Conclusion:** These preliminary data confirm that ESBL-producing *E. coli* of the CTX-M type have emerged as important pathogens in urinary tract infections also of outpatients in both regions. As the only available study from North Tyrol (2008) found 1% CTX-M in *E.coli* from mostly hospitalized patients this suggests that there has been a shift in genotypes and/or the observed increase 2002-7 in TILAK hospitals may have been caused by strains different from those found in outpatients?

As the mortality rate of blood stream infections with ESBL producers is double of that of non producers according to meta-analysis, we should keep an eye on them.

## 4. Novel PKCθ/Nedd4/Cbl-b Complex in T cells – Biological Role and Mode of Action

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**Background:** Our aim is to elucidate the non-redundant antagonism between PKCθ mediated Cbl-b phosphorylation and Nedd4-dependent Cbl-b ubiquitination that regulates TCR/CD28 activation thresholds in primary T cells.
**Methods:** To unravel the antagonism between PKCθ and Cbl-b mice deficient in either one or both genes were used. Rescue studies were performed by proliferation and cytokine release assays. Cbl-b ubiquitination was performed by transfection of Jurkat cells with plasmids containing PKCθ or Cbl-b together with ubiquitin expression vectors. To confirm the in-silico predicted PKCθ phosphorylation site on Cbl-b, a protein kinase assay was performed. To prove phosphorylation on S282 endogenously a (p)site-specific Antibody was raised. To show Cbl-b/Nedd4 interaction co-immunoprecipitation analysis was performed.
**Results:** Analysis of the activation thresholds of PKCθ and Cbl-b single and doubly deficient T cells showed that concomitant loss of Cbl-b substantially restored the defective proliferation and IL-2 responses of PKCθ deficient T cells. Consistent with our model of PKCθ being a negative regulator of Cbl-b, the abundance of ubiquitinated Cbl-b was substantially enhanced in Jurkat T cells that contained a constitutively active mutant of PKCθ. PKCθ phosphorylated S282 on Cbl-b efficiently in vitro and this phosphorylation could be confirmed endogenously in primary T cells. In regard to our future working model, the E3 ligase Nedd4 physically interacts with Cbl-b upon CD3/CD28 costimulation independently of PKCθ.
**Conclusions:** This study defines an essential and non-redundant regulatory function of PKCθ in the ubiquitination and subsequent degradation of Cbl-b as a prerequisite for a productive immune response. To elucidate the physiological role of S282 and its contribution of PKCθ mediated Cbl-b phosphorylation on subcellular localization, signaling complex formation and Nedd4 dependent Cbl-b ubiquitination, remain to be resolved in our future studies.

## 5. Novel PKC/NR2F6/NFAT Signaling Pathway Determines Immune Response Thresholds of $CD4^+$ T cells

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PKC signaling to NFAT/AP-1 transactivation critically involves the recently identified NR2F6, a nuclear orphan receptor predominantly expressed in the $CD4^+$ T helper cell (Th)17 subset (Hermann-Kleiter et al., Immunity 2008). Mechanistically, PKC-mediated phosphorylation on Ser 83 within the DBD-domain of NR2F6 results in the release of NR2F6 from its DNA enhancer sites within the Il17a promoter, as revealed by EMSA and ChIP analysis. NR2F6 potently antagonizes the ability of Th17 $CD4^+$ T cells to induce expression of key cytokines such as IL-17, IL-23, and IL-21. In Th17 cells differentiated and activated ex vivo, loss of Nr2f6 results in amplified NFAT DNA binding at the Il17a promoter and subsequently increased IL-17 transcription. Consistently, Nr2f6-deficient mice have hyper-reactive lymphocytes and develop late-onset immune-pathologies and are hypersusceptible to the Th17-dependent model of experimental autoimmune encephalomyelitis.
Taken together, our study establishes NR2F6 as PKC substrate and critical transcriptional repressor of autoimmunity.

## 6. Decreased $CD4^+$ naive T-cells in Children after Thymectomy and delayed Antibody Response to a New Antigen

M. Zlamy[1], C. Gögele[1], R. Würzner[2], R. Geiger[3], L. B. Zimmerhackl[1], M. Prelog[1]

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**Background:** The study was designed to assess indicators of immunosenescence, such as proportions of peripheral blood naive T cells, the T cell receptor excision circles (TRECs) and Ki67-expression as a marker of thymic function and peripheral replication in thymectomized patients (TP) compared to healthy age-matched donors (HD). The second aim of our study was to investigate whether children after thymectomy may show a poor antibody response to new antigens like vaccines.

**Methods:** T cells of 101 TP and 81 HD were gated for naive ($CD4^+CD45RA^+CD62L^+$) and memory T cells ($CD28^+CD45RO^+$) by flow cytometry. Forty-four TP and 56 HD were vaccinated with tick-borne encephalitis virus (TBEV) vaccine (FSME Immun junior, Baxter, Vienna, Austria) following the standard 3 dose vaccination schedule. IgG levels were evaluated each 4 weeks after the second and third vaccination. Testing of TBVE IgG levels and IgG avidity with a commercial test kit (Euroimmun, Lübeck, Germany) was performed 3 years after the first vaccination.

**Results:** TP showed decreased naive T cell counts compared to HD ($p<0.001$) and demonstrated significantly lower TREC numbers in naive T cells ($p<0.001$). TREC numbers significantly correlated with time post thymectomy ($p<0.001$). Higher percentages of Ki67-expresssing naive T cells were found in TP compared to HD ($p<0.01$). TP showed 2.2-fold lower TBEV IgG antibody levels after the second vaccination when compared to HD ($p=0.03$), but a normal response after the third vaccination.

Two years after the third vaccination, there was neither a statistical difference when comparing IgG antibody levels of TP and HD, nor any differences according to avidity.

**Conclusion:** The findings indicate that changes of the peripheral naive T cell subset in TP may resemble the findings of an aging immune system in elderly persons after thymic involution. Our data provide evidence that peripheral T cell homeostasis in TP is maintained mainly by extrathymic expansion of existing naive T cells in the periphery to compensate the diminished thymic output. Our results also showed a delayed increase of TBEV IgG antibody levels after vaccination in thymectomized children. This may indicate alterations of the primary T cell immune response to new antigens but a normal memory function. Thus, it seems mandatory to monitor antibody responses to other vaccinations as well as infection rates in thymectomized children to avoid long-term complications.

## 7. Regulatory Role of CYTIP in Mouse Dendritic Cells

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Cytip (Cytohesin 1 Interacting Protein) was suggested to be involved in the immunological synapse formation and duration of T cell and dendritic cells (DC) contact by modulating the binding between LFA-1 and ICAM-1.

To investigate the role of the intracellular molecule Cytip in the immune system, we analysed spleen and lymph node cells from Cytip ko and wildtype mice by FACS analysis regarding their numbers of T and B cells and different DC populations. We found out that Cytip ko mice have less $CD4^+$ T cells in spleen and lymph nodes but only less $CD8^+$ T cells in the spleen in comparison to their wildtype counterparts. In contrast to the number of T cells, there are more B cells in the spleen of Cytip ko mice. No difference could be found in the lymph nodes concerning the different dendritic cell populations (Langerhans cells, Langerin+ dermal Dendritic cells, dermal DC).

To further investigate the role of Cytip in DC we used ovalbumin loaded BMDC (bone marrow derived denritic cells) as antigen presenting cells (APC) in a mixed lymphocyte reaction (MLR) with either OT-I or OT-II T cells. In both MLRs we measured higher proliferation when BMDC generated from Cytip ko mice were used as APC.

To explore the function of Cytip in DC in vivo, we used TNBS modified bone marrow derived DC from wildtype and ko mice and injected them intradermally into wildtype recipients. After 5 days mice were challenged with 1% TNCB on both sides of the ear and ear swelling was measured. In wildtype mice sensitised with TNBS treated Cytip ko BMDC, ear swelling reaction was much more pronounced than in those mice treated with wildtype BMDC.

In addition to these results we explored also the role of Cytip in DC during the challenge phase of contact hypersensitivity reaction. For this, we sensitised wildtype mice with 1% TNCB on the shaved abdomen and transferred spleen cells from these mice into wildtype and Cytip ko mice 5 days after sensitisation. Mice were challenged with TNCB 2h after spleen cell transfer and again ear swelling reaction was measured. Also under these conditions, we found higher ear swelling in Cytip ko mice.

From these in vitro and in vivo results we speculate that Cytip might have a regulatory function in mouse Dendritic cells, although the molecular mechanism is still elusive.

## 8. Pregnane X Receptor (PXR) Signalling Links Drug Metabolism to the Cutaneous Adaptive Immune Response

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The pregnane X receptor (PXR) is a ligand-activated transcription factor regulating genes central to drug and hormone metabolism in the liver. Previous reports indicated that PXR is expressed in PBMC, but the role of PXR in immune cells remains unknown. We here report increased PXR expression in mouse and human T-lymphocytes upon immune activation. Furthermore, pharmacologic activation of PXR inhibits T-lymphocyte proliferation, and anergizes T-lymphocytes by decreasing the expression of CD25 and IFN-gamma and decreasing phosphorylated NF-kappaB and MEK1/2. While these effects are preceded by an increase of SOCS-1, a master switch for IFN-gamma expression, in a PXR-dependent manner, T-bet expression remains unchanged. Conversely, PXR deficient mice exhibit an exaggerated T-lymphocyte proliferation and increased CD25 expression. Furthermore, PXR deficient lymphocytes produce more IFN-gamma and less of the anti-inflammatory cytokine IL-10. In summary, these results reveal a novel immune-regulatory role of PXR in T-lymphocytes and identify SOCS-1 as an early signal in PXR-mediated T-lymphocyte suppression.

## 9. PPAR-alpha deficiency triggers allergic contact dermatitis by affecting regulatory T cells via lack of IL-2

S. Dubrac[1], A. Elentner[1], K. Schoonjans[2], J. Auwerx[2], and M. Schmuth[1]
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The aim of our work was to decipher the cellular basis of the pro-inflammatory skin phenotype of PPAR-alpha deficient mice. After challenge with a contact allergen, contact hypersensitivity reaction was increased and prolonged in PPAR-alpha deficient mice when compared to wild type mice. Numbers of T-lymphocytes in the skin of PPAR-alpha deficient mice were increased and showed enhanced expression of the activation marker CD25 when compared to controls. After antigen challenge, percentages of Treg in the blood, the skin draining lymph nodes and the skin were decreased in PPAR-alpha deficient mice when compared to controls. Moreover, PPAR-alpha deficiency impaired the production of IL-2 in lymph nodes, whereas production of TGF-beta remained unchanged. Injection of PPAR-alpha deficient mice with IL-2 restored the size of the Treg population in the skin draining lymph nodes of challenged mice. In vivo induction of Treg from wild type $CD4^+CD25^-$ T-cells was impaired when adoptively transferred into PPAR-alpha deficient mice as compared with wild type mice and reversed by injection of IL-2. Furthermore, PPAR-alpha deficient Treg exhibited impaired suppressive capacity when compared to wild type Treg in mixed leukocyte reactions. Co-adoptive transfer of both $CD4^+$ T cells and Treg confirmed poor suppressive capacity of PPAR-alpha deficient Treg in vivo presumably due to reduced IL-10 and perforin/granzyme B. Injection of IL-2 fully restored the expression of perforin in PPAR-alpha deficient mice but partially the expression of granzyme B. In conclusion, PPAR-alpha deficiency aggravates skin contact hypersensitivity by affecting Treg function through lack of IL-2.

## 10. The Role of Cutaneous Dendritic Cells in Skin Cancer

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Dendritic cells are specialized antigen presenting cells that are crucial for the induction of immunity and tolerance. The unique features of dendritic cells are the uptake, processing and presentation of antigens to naive T cells in lymphatic tissue. The activated T cells home to peripheral tissue, such as the skin, where they kill microbes, infected cells and tumor cells. The skin harbours two major subsets of dendritic cells, the Langerhans cells in the epidermis and the dermal dendritic cells in the dermis. In the event of infection, dendritic cells migrate from the skin to the draining lymph nodes where they activate T cells. Dendritic cells are widely used for immunotherapy of various tumors and have proven to be safe for the patients and to induce cytotoxic T cell responses against the tumor. The preparation of the dendritic cell vaccine is cumbersome and the efficiency has to be improved since very few patients show partial or complete remission. Langerhans cells so far were thought to be major players in skin immunity, however, in the last few years doubts were raised about their importance in vivo. Little is known about the role of dermal dendritic cells and the recent discovery of a third population of skin DC, the dermal langerin+cells, shows how complex the skin immune system is. Although we know from our own work that Langerhans cells are very potent antigen-presenting cells and are crucial for the induction of T cell responses, the role of skin dendritic cells in immune responses against cutaneous tumors remains elusive. In our project we are investigating the specific role of cutaneous dendritic cells in skin cancer and intend to design novel improved immunization strategies by exploiting the therapeutical potential of skin dendritic cells. This work is important because we need to understand the skin immune system to optimally harness the unique immunogenic properties of skin dendritic cells to treat cancer.

## 11. Decreased Antibody Titers and Booster Responses in Tick-borne Encephalitis Vaccinees Aged 50 to 90 Years

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**Background:** The flavivirus Tick-borne encephalitis virus (TBEV) is endemic in parts of Europe and Asia and is transmitted mainly by ticks. 20-30% of infected persons experience CNS symptoms, such as meningitis or meningoencephalitis and permanent neurological sequelae are frequently observed. Vaccination with a purified, formalin-inactivated whole virus vaccine is highly efficient to prevent disease and is recommended in endemic areas. Because of decreased immune functions of older people booster intervals of 3 years – instead of 5 – are recommended for TBE vaccinations for persons ≥60 years in Austria. So far, no comparative data on the immune-responsiveness of the age group 50 to 59 years are available.

**Methods:** We investigated the antibody titers and booster responses (in ELISA and neutralization assays) for the age groups 50-59, 60-69, and >69 years in comparison to a control group below 30 years.

**Results:** Pre-and post-vaccination antibody concentrations as well as neutralizing antibody titers were highest in the young age group but significantly lower in all other age groups. Notably, there was no difference between individuals aged 50 to 59 years compared to older groups. Antibody concentrations were lower 5-7 years after the last vaccination compared to 3-4 year intervals in persons older than 60 years. Nevertheless, antibodies were still detectable and could be sufficiently increased by booster shots in the vast majority of persons. However, four persons above the age of 60 did not have detectable neutralizing antibody titers despite primary vaccination 15-20 years earlier and regular booster vaccinations thereafter. Antibodies could be induced by booster vaccination in these persons, remained however considerably lower than the mean post-vaccination values for this age-group.

**Conclusion:** Our results indicate that the responsiveness of the immune system to vaccination is already impaired at the age of fifty. Careful considerations will be needed to adequately balance the wish for longer booster intervals, which means fewer shots combined with lower costs, and the risk of a few persons with low antibody responses to develop disease.

## 12. The Bone Marrow Environment Maintains Polyfunctional Memory T cells During Aging

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The bone marrow (BM) has recently been attributed a key role in memory T cell maintenance. However, little is still known about the distribution, phenotype and function of human T cell subsets in the BM and how aging affects BM T cell function and survival. Here we show that human BM T cells are in a heightened activation state, express a characteristic set of chemokine- and co-stimulatory receptors compared to peripheral blood (PB) and have a partly distinct T cell receptor repertoire. The number of polyfunctional $CD8^+$ T cells was higher in the BM compared to the PB. Aging led to a decline of naive T cells and to an accumulation of cells with an effector-memory phenotype in the BM. Though, the age-related increase in highly differentiated CD57-expressing effector $CD8^+$ T cells was much lower in the BM compared to the PB. Importantly, the frequency of polyfunctional BM $CD8^+$ T cells was maintained during aging. The expression of the pro-inflammatory cytokine IL-6 was increased during aging in the BM and gene array analysis revealed a network responsible for the age-related increase in IL-6. In conclusion, our results indicate that the BM environment is important for the maintenance of polyfunctional memory $CD8^+$ T cells during human aging.

## 13. Intracellular Tetrahydrobiopterin Concentrations Modulate Alkylglycerol Monooxygenase Activity in Intact RAW 264.7 cells

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**Background:** (6R)-5,6,7,8-Tetrahydrobiopterin is an essential cofactor for the aromatic amino acid hydroxylases (phenylalanine, tyrosine, and tryptophan hydroylases), the nitric oxide synthases (NOS) and alkylglycerol monooxygenase. It has been described that the treatment of cells with tetrahydrobiopterin enhances cell proliferation. This activation of proliferation could not be assigned to the action of nitric oxide synthases and aromatic amino acid hydroxylases since the effect has also been described in cells that lack corresponding enzymatic activity.
The tetrahydrobiopterin dependent enzyme alkylglycerol monooxygenase is so far the only enzyme known that is able to degrade ether lipids and thereby prevents their accumulation in cells. Beside anti-tumor effects, antibiotic-like acitivity and immunostimulation, it has been described that ether lipids have an inhibitory effect on the activity of protein kinase C, which is a regulator of cell proliferation. The aim of this study is to investigate whether intracellular tetrahydrobiopterin influence ether lipid cleavage in RAW 264.7 cells.
**Methods:** In our experiments tetrahydrobiopterin synthesis was repressed in RAW 264.7 cells by the addition of 2,4-Diamino-6-hydroxypyrimidine (DAHP), which is a specific inhibitor of GTP cyclohydrolase I. This inhibition was reconstituted by feeding cells with sepiapterin which is metabolized into tetrahydrobiopterin by sepiapterin reductase and dihydrofolate reductase. Alkylglycerol monooxygenase activity was measured in vivo by incubating cells with 5 µM 1-O-pyrenedecyl glycerol substrate and measuring supernatant with our HPLC system and fluorescent detection. The enzymatic activity was quantified by the amount of pyrenedecanoic acid measured after an incubation time of 2h, 6h, 10h and 24h. Cell proliferation was measured using the Cell Proliferation Kit I (MTT) (Roche Diagnostics GmbH, Mannheim, Germany). Tetrahydrobiopterin concentration in cells were measured and served as control for the efficiency of inhibition.
**Results:** Low intracellular tetrahydrobiopterin levels reduced the enzymatic activity of alkylglycerol monooxygenase in RAW 264.7 cells. At least 3 mM DAHP is necessary to achieve sufficient inhibition of tetrahydrobiopterin synthesis in order to reduce alkylglycerol monooxygenase activity. The addition of 0 – 50 µM sepiapterin showed that 1 µM was enough to fully recover enzymatic activity.
**Conclusion:** In this work we demonstrate that the intracellular tetrahydrobiopterin level controls the extent of ether lipid cleavage by alkylglycerol monooxygenase.

## 14. Nifedipine Affects the Course of Salmonella Infection by Pharmacological Modification of Iron Homeostasis

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**Background**: Iron overload aggravates the clinical course of infections by negatively affecting cell mediated immune mechanisms of macrophages and T helper cell type 1 effector pathways. Recently, we have shown that the calcium antagonist nifedipine enhances DMT1 mediated iron transport *in vitro* and causes subsequent depletion from liver and the circulation in iron overloaded mice. Herein, we thus investigated whether nifedipine may impact on the clinical course of an invasive infection via modulation of iron homeostasis.

**Methods**: RAW 264.7 cells were infected with *Salmonella thyphimurium* and stimulated with varying concentrations of nifedipine. Mice were fed with a control diet or iron enriched diet for three weeks, infected intraperitoneally with *Salmonella thyphimurium* and treated with solvent control or nifedipine for three consecutive days. 24 hours later mice were sacrificed, the bacterial loads were quantified in the liver and the spleen, and the expression of iron regulating genes was determined by means of RT-PCR and Western blot analysis.

**Results**: The cell culture model revealed a significant decrease of the bacterial load upon nifedipine treatment. In RT-PCR analysis an increase of the iron exporter ferroportin1 expression was induced in the nifedipine treated group.

Nifedipine treated mice, independently of dietary iron overload, showed an improved survival and presented with reduced numbers of Salmonella in the liver and the spleen as compared to solvent injected animals. Even though these effects were more pronounced in the iron diet fed group. Serum iron levels were decreased as a reflection of the iron depleting effects of the drug. This was paralleled by decreased ferritin levels in the liver and spleen and increased ferroportin 1 expression by means of western blot analysis. Interestingly, we did not observe differences in the expression of a panel of pro- and anti-inflammatory cytokines according to nifedipine treatment.

**Conclusion**: Our data provide evidence that nifedipine may be a promising adjunctive therapy for the treatment of infections with intracellulare pathogenes. Mechanistically the iron depleting effects may refere to the increase of Fp expression as a consequence of DMT-1 induction by nifedipine.

## 15. N-Chlorotaurine, a Product of Activated Leukocytes, Can be Used as an Antiseptic in Human Medicine

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**Background:** N-chlorotaurine (NCT), the N-chloro derivative of the amino acid taurine, is produced from taurine and hypochlorous acid formed by myeloperoxidase during the oxidative burst of granulocytes and monocytes. As a long-lived oxidant with mild activity, it has been considered as an antimicrobial agent for different body regions in human (and veterinary) medicine.
**Methods:** Antimicrobial activity has been investigated largely with quantitative killing assays in buffer solution and body fluids. Clinical trials of phase I and II as well as animal tests have been performed to test in vivo tolerability and efficacy.
**Results:** Microbicidal activity of a 1% (55 mM) aqueous and buffered solution has been found against all tested strains of Gram-positive and Gram-negative bacteria (e.g. staphylococci, streptococci, enterobacteriaceae, Pseudomonas aeruginosa), of viruses (adenovirus, herpesvirus 1 and 2, HIV, and influenza), of fungi (Candida spp. and moulds), and of protozoa (acanthamoebae, leishmaniae, trichomonads). A postantibiotic effect after sublethal incubation times and enhancement of the activity in the presence of organic material, particularly ammonium, are special phenomena.
Clinical studies demonstrated very good tolerability of 1% NCT at different body sites (eye, skin, outer ear canal, nasal cavity, paranasal sinuses, oral cavity, urinary bladder, vagina etc.) and up to date efficacy in conjunctivitis, crural ulcers, and otitis externa. In a porcine model and in a pilot study in man, inhalation of 1% NCT was very well tolerated. Recently synthesized dimethylated derivatives of NCT, which can be long-term stored at room temperature, may have similar properties.
**Conclusion:** NCT is an endogenous antiseptic very well tolerated, but sufficiently efficacious at different body sites. Because of these properties, it seems to be useful also for delicate regions like the bronchopulmonary system. NCT derivatives with higher stability are of high interest, too.

## 16. Tim-3 is Upregulated and Protective in Nephrotoxic Serum Nephritis

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**Background:** T cell immunoglobulin and mucin protein-3 (Tim-3) is mainly expressed on the cell surface of T-helper lymphocytes ($T_H$) that negatively regulates $T_H$-type 1 ($T_H$-1) responses. Since blockade of Tim-3 aggravated disease activity in $T_H$-1 dependent diseases, we investigated whether Tim-3 is involved in the pathogenesis of the $T_H$-1-dependent nephrotoxic nephritis (NTS).

**Methods:** 8 BALBc mice were pre-immunized subcutaneously with 2 mg/ml rabbit IgG dissolved in incomplete Freund´s adjuvant and non-viable desiccated Mycobacterium tuberculosis H37a. After 3 days, heat-inactivated rabbit anti-mouse GBM antiserum was injected via the tail vein. Mice received 150µg anti-TIM-3 blocking antibody or a rat IgG isotype control antibody intraperitoneally on the day of immunization. Three days later mice received an additional dose of 50µg anti-TIM-3 blocking antibody or solvent intraperitoneally. The animals were sacrificed after 14 days after initiation of the NTS. We extracted RNA and protein from kidneys and lymph nodes and studied the expression of critical genes in innate and aquired immunity by means of QRT-PCR and Western blots. Urinary albumin, creatinine as well as lipocalin were carried out by ELISA. Formalin-fixed renal tissue was embedded in paraffin for immunohistochemistry. Single cell suspensions of kidneys were performed for FACS analysis.

**Results:** We first evaluated Tim-3 expression in mice after induction of nephrotoxic serum nephritis (NTS) and then studied the effects of anti-Tim-3 treatment towards the course of NTS for up to seven days. While Tim-3 expression was undetectable in control mice, we found significantly increased Tim-3 expression in kidneys, but not in draining lymph nodes, at one, four and eight weeks after induction of NTS. Tim-3 expressing cells infiltrating kidneys of mice subjected to NTS turned out to be $CD4^+$ T cells rather than $CD8^+$ cytotoxic T cells and dendritic cells. Administration of a blocking anti-Tim-3 antibody aggravated nephritis as shown by significantly increased albuminuria, respective histological changes and increased expression of the kidney injury molecule lipocalin-2. In parallel, an increase of infiltrating T cells, macrophages and macrophage pro-inflammatory cytokine formation as well as increased proliferation and apoptosis in kidneys of anti-Tim3 treated mice was detected.

**Conclusion:** Together, we provide first evidence that Tim-3 is up-regulated in kidneys in NTS, and that Tim-3 exerts protective roles in the course of disease.

## 17. Conditional Gene Ablation of the MAP Kinase Adapter Protein p14 in Dendritic Cells leads to Severe Disturbance of Tissue Homeostasis

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**Background:** Dendritic cells are key players of the immune system and link innate to adaptive immune response. Their major task is to take up pathogens, process them and present the antigen to T cells. These processes are strongly dependent on endosomal/lysosomal trafficking. Conditional gene disruption of the adapter protein p14 in mice demonstrates that the late endosomal p14/MP1-MEK1 signaling complex is required to control endosomal traffic and tissue homeostasis (Teis et al., J Cell Biol, 2006).
Methods: To address the molecular function of p14 in dendritic cells, we generated a conditional knock out mouse model which allowed us to specifically delete p14 in CD11c expressing cells. The effects were analyzed in tissue (histological methods) and primary cell culture (FACS-Analysis, Western Blot).
**Results:** The mice were viable and developed a severe pathological phenotype at the age of three months. The most obvious morphological characteristics included enlarged lymph nodes and splenomegaly. The structural integrity of these organs was disarranged and massive leukocyte infiltrates were observed. Furthermore, these mice developed infiltrates of monocytes and dendritic cells in skin and liver. The bone marrow of the CD11c-p14 knock out mice was hyperplastic, accompanied by an increase of hematopoetic stem cells. Furthermore a shift from the granulocytic towards the monocytic lineage, an increase in the T helper cell population and a decrease of the erythrocyte progenitors was observed. Bone marrow derived dendritic cells showed an increased expression of maturation markers after stimulation with Lipopolysaccharide. Immature dendritic cells were able to take up latex beads and yeast to late endosomal compartments but showed an impaired ability to migrate.
**Conclusion:** Taken together, p14 severely affects the tissue homeostasis of dendritic cells. Further immunological and cell biological investigations will help us to elucidate the role of p14 in dendritic cells.

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## 18. Clonotypic Archetypes in the T Cell Repertoire Mark Persistent Oligoarticular Juvenile Idiopathic Arthritis

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**Background:** Juvenile Idiopathic Arthritis (JIA) is characterized by chronic inflammation. Diagnostic markers for JIA have not been established yet. T cells are supposed to play an important role in the pathogenesis of JIA. The aim of this study was to demonstrate a profile of a limited T cell repertoire in the peripheral blood (PB) and the synovial fluid (SF) of JIA patients with persistent oligoarthritis (oJIA).

**Material and Methods:** Paired samples from PB and SF of 9 patients with oJIA were analyzed for their T cell repertoire based on the Vbeta-chain variability of the T cell receptor (TCR). Multicolour flow cytometry was performed using a panel of TCR Vbeta and CD4 or CD8 specific monoclonal antibodies.

**Results:** The CD4/CD8 ratio was inconspicuous in both compartments. Comparing CD4 and CD8 cells differences were observed in both compartments with predominance of Vbeta 2, 5.1 and 17 in PB and V Vbeta 2 and 5.1 in the SF CD4 cells. In CD8 cells Vbeta 2, 3, 13.1 and 13.2 were dominant in the PB and Vbeta 1, 2, 12 and 13.2 in the SF. Double positive T cells present significantly Vbeta 13.1 in PB mononuclear cells and in SF.

**Conclusion:** Expression patterns of Vbeta chains suggest oligoclonal T cell expansion of Vbeta families differing in PB and SF in patients with persistent oJIA. Further studies are necessary to evaluate, if these patterns characterize persistent oJIA.

## 19. Functional Analysis of the Complement System in Oligoarticular Juvenile Idiopathic Arthritis

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**Objective:** Juvenile idiopathic arthritis (JIA) summarizes a group of chronic joint diseases in childhood. The role of the complement sytem in the pathogenesis of JIA is unclear. The aim of this study was to evaluate the contribution of the complement system in the pathogenesis of oligoarticular JIA.

**Material and Methods:** Serum of the peripheral blood and the synovial fluid were investigated for activity of the classical pathway (CP), the mannose binding lectin (MBL) pathway and the alternative pathway (AP) of the complement system.

**Results:** A total of 12 samples from PB from two girls and two samples from SF from two joints of one girl (four and five years old) with oligoarticular JIA were investigated in a longitudinal observation from the timepoint of the diagnosis of JIA. The differences between the complement activity in the PB and in the SF were extremly statistically significant (CP and MBL: $p < 0.0001$; AP: $< 0.0087$). The results for CP and the MBL pathway were considered to be pathologic.

**Conclusion:** The AP is the main contributor in the pathogenesis of oligoarticular JIA. Anti C5 therapy may be an option to avoid the creation of the membrane attack complex.

## 20. Harnessing the Immune System for Targeted Cancer Therapy

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The Cell Therapy Unit (CTU) is part of Area 1 (Mechanisms controlling Tumor Growth and anti-Tumor Immunity) within the Oncotyrol research consortium. It is jointly operated by researchers from the Departments of Urology and Dermatology.
CTU develops effective and well tolerated immunotherapeutic approaches based mainly on *dendritic cells* to control and / or eliminate human cancers. In addition, CTU characterizes and validates anti-tumor immune responses. The efficacy of the final cancer vaccine consisting of tumor antigen(s) and adjuvant will be assessed in clinical trials.
Dendritic cells are either isolated directly from blood or generated in vitro from precursor cells in a two-step culture system, which is being used in clinical trials. Efforts are directed towards improving the efficacy of “classical” monocyte-derived dendritic cells in inducing cytotoxic T cells. Such work will include the fine-tuning of dendritic cell generation, maturation and migration as well as antigen loading and clinical administration. Regulation of dendritic cell signal strength is examined to generate approprate memory resonses in order to achieve long-term protection. Novel subsets of directly blood-derived dendritic cells, defined by their expression of CD56, are characterized both phenotypically and functionally. Importantly, their capability to activate innate effector lymphocytes including NK cells and $\gamma\delta$ T cells is studied. The immune-enhancing properties of targeting antigens to endocytic receptors (e.g., DEC-205) are being explored in human skin, based on recent data corroborated in mouse models.
Research of CTU will result in the development and clinical implementation of broadly applicable cancer vaccines that lead to increased cancer patient survival without impairing the patients' quality of life.

## 21. Improvement of Intradermal Immunization against Melanoma

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**Introduction:** Gycolipid antigens are currently tested as adjuvant for immunotherapy as they are able to enhance T cell responses after being presented by dendritic cells to natural killer T cells. We were interested in examining the potential of glycolipid antigen as adjuvant for skin immunization against melanoma. In addition, we investigated if targeting antigen to skin dendritic cells with an antibody would improve T cell responses.

**Methods:** We measured T cell responses after intradermal immunization of mice with the synthetic glycolipid alpha-Galactosylceramide (alpha-GalCer) plus the model antigen Ovalbumin protein (OVA) or OVA conjugated to an antibody against the surface molecule DEC-205/CD205. OVA was used together with alpha-GalCer for immunization against murine OVA-expressing B16-melanoma (B16.OVA). The involvement of skin dendritic cells in this process was tested by removal of the immunization site and with transgenic mice.

**Results:** Intradermal immunization with alpha-GalCer plus OVA strongly enhanced endogenous $CD8^+$T cell responses. As a consequence the growth of transplanted B16.OVA melanoma cells was inhibited in mice. Skin dendritic cells were not involved since depletion of skin dendritic cells did not alter cytotoxic immune responses after intradermal immunization with alpha-GalCer and OVA. Targeting the same antigen to skin dendritic cells with an antibody against DEC-205 allowed using 1000-times less antigen to obtain similar inhibition of tumor growth.

**Conclusion:** Thus, the glycolipid alpha-GalCer is a useful adjuvant for intradermal immunization strategies and in combination with targeting of antigen to skin dendritic cells inhibits tumor growth even with small amounts of antigen.

## 22. The Lambaréné-Organ-Dysfunction Score (LODS) is a Simple Clinical Predictor for Fatal Malaria in African Children

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**Background:** *Plasmodium falciparum* malaria accounts for more than a million deaths annually, mostly among young children in sub-Saharan Africa. Identifying those who are likely to die is crucial. Several factors have been independently associated with mortality. As malaria is a systemic disease, a quantitative score, combining such risk factors may be superior.

**Methods:** We used both, forward and backward stepwise logistic regression to select the best predictors for death evaluated on in 23,890 African children with severe *Plasmodium falciparum* malaria. The study was conducted from December 2000 to May 2005 in six hospital-based research units (Banjul in The Gambia, Blantyre in Malawi, Kilifi in Kenya, Kumasi in Ghana and Lambaréné and Libreville in Gabon) in a network established to study severe malaria in African children (SMAC).

**Results:** The Lambaréné-Organ-Dysfunction-Score (LODS) combines three variables: coma, prostration and deep breathing. A LODS > 0 (OR = 9.6; 95%CI 8.0-11.4) has a sensitivity of 85% to predict death and a LODS < 3 is highly specific for survival (98%).

**Conclusion:** The LODS is a simple clinical predictor for fatal malaria in African children. This score provides an accurate and rapid identification of children needing either referral or increased attention.

## 23. The Role of the Heat Shock Protein 90 in Amphotericin B Resistant *Aspergillus Terreus*

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Invasive aspergillosis (IA) is a leading cause of mortality among immunocompromised individuals and the frequency of *Aspergillus* spp. infections has increased in recent years [1]. *Aspergillus fumigatus* (*A. fumigatus*) is the most common pathogen involved in IA, but at the Innsbruck Medical University the number of infections due to *Aspergillus terreus* (*A. terreus*) are increasing. *A. terreus* is a particular Amphotericin B (AmB) resistant fungus. This study determined the role of the essential molecular chaperones Hsp90 and Hsp70 in AmB action [2].

Therefore each of two strains, *A. terreus* and *A. fumigatus* were investigated in detail. Susceptibility testing for Amphotericin B was performed according to E-test-method; *A. terreus* and *A. fumigatus* showed MICs > 32 µg/ml and MICs ≤ 1 µg/ml, respectively. Hsp90 was blocked with the inhibitor Geldanamycin (5 µM, 10 µM and 20 µM). The results indicate that the two *A. terreus* became highly sensitive to AmB, as MICs decreased to < 1 µg/ml AmB. Blocking Hsp90 in *A. fumigatus* decreased MICs to < 0.13 µg/ml AmB. Hsp70 was blocked via KNK423 (5 µM, 10 µM and 20 µM) addition to the agar; no effect in susceptibility of *A. terreus* and *A. fumigatus* to AmB has been observed; the AmB-MICs were still > 32 µg/ml and ≤ 1 µg/ml, respectively.

To detect Hsp90 gene expression, Northern Blot analysis were performed for *A. terreus* and *A. fumigatus* with sublethal concentrations of 2 µg/ml AmB and 0,25 µg/ml AmB, respectively. The results indicate an upregulation of Hsp90 gene in *A. terreus* but not in *A. fumigatus*.

Taken together, these data provide first direct evidence that Hsp90 may be responsible for the AmB resistance in *A. terreus*.

1. Maschmeyer, G., Haas, A., Cornely, O.A., Invasive aspergillosis: epidemiology, diagnosis and management in immunocompromised patients. Drugs 2007, 67, 1567 - 1601

2. G. Blum, P. Gruber, S. Perkhofer, B. Sarg, H. Lindner, M. Nagl, M. P. Dierich, C. Lass-Flörl; Amphotericin B induces heat shock proteins in polyene resitant *Aspergillus terreus;* Department of Hygiene, Microbiology and Social Medicine, Innsbruck Medical University; submitted;

## 24. SCR1/2 of C4bp Induces Lysis of N. Gonorrhoeae

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**Background:** C4b-binding protein (C4bp), a regulator of complement activation (RCA) is attached to the surface of N. gonorrhoeae thereby protecting the bacteria against complement-mediated lysis. At the bacterial surface, Porins are supposed to be involved in these interactions. The putative binding site of C4bp is thought to be located within SCR 1/2. We tested whether this SCR binds to the bacterial protein, thus preventing the interaction of native C4bp and favouring lysis of N gonorrhoeae by human complement.
**Methods:** To harvest sufficient amounts of C4bp-derived SCR, the sequences were expressed in the Pichia pastoris system and purified by affinity chromatography via the HIS-tag of the SCRs. Lysis assays were perform to test inasmuch SCR1/2 of C4bp can be used as anti-bacterial compound.
**Results:** Western blot analysis verified the expression of C4bp-SCR1/2. As shown by heparin binding experiments, the purified protein was functional active. In lysis experiments we were able to induce a significant decrease of the bacterial colonies in the presence of SCR1/2.
**Conclusion:** SCR1/2 may provide an alternative approach for the therapy of N. gonorrhoeae infections.

## 25. Improving Antigen Presentation by Targeting Antigens to CD11c on Dendritic Cells

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**Background:** Previous results demonstrated that complement serves as endogenous adjuvant to enhance retrovirus-specific CTL responses induced by dendritic cells. Therefore targeting CD11c, the alfa-subunit of complement receptor type 4 (CR4) on dendritic cells (DC) might provide a potential vaccination tool to improve antigen presentation.
**Methods:** We have chosen Friend Virus (FV), a mouse retrovirus representing a well established model to investigate novel vaccination strategies. For targeting CD11c, we generated single-chain mAb fragments (scFv) fused to immune-dominant region (IDR) of FVgag.
Results: CD11c-scFv-IDRgag constructs were tested for binding to DCs. Compared to control-scFv-IDRgag, DCs targeted in vitro with CD11c-scFv-IDRgag constructs showed significantly higher capacity to activate FV-specific TCR transgenic CD8 T cells. Furthermore, FV-specific CD8 T cells activated by CD11c-scFv-IDRgag-loaded DCs showed significantly pronounced proliferation and killing of FV-infected target cells.
**Conclusion:** Further experiments are necessary to prove the efficacy of CD11c-scFv-IDRgag to induce specific CTL response in vivo.

## 26. EspP, a Serine Protease of Enterohemorrhagic Escherichia coli, Impairs Complement Activation by Cleaving Complement Factors C3/C3b and C5

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Hemolytic uremic syndrome (HUS) is a life-threatening disorder characterized by hemolytic anemia, thrombocytopenia, and renal insufficiency. It is mainly caused by infections with enterohemorrhagic Escherichia coli (EHEC). Recently, Shiga toxin 2, the best studied virulence factor of EHEC, was reported to interact with complement, implying that complement is involved in the pathogenesis of EHEC-induced HUS.
The aim of the present study was to investigate whether or not the serine protease EspP, an important virulence factor of EHEC, interacts with complement proteins.
EspP did not have any effect on the integrity of Factor H or Factor I. However, EspP was shown to cleave purified C3/C3b and C5. Cleavage of the respective complement proteins also occurred in normal human serum as source for C3/C3b or C5 or when the purified complement protein was added to supernatant of an EspP producing wildtype strain. Edman degradation allowed unequivocal mapping of all three main C3b fragments, but not of the three main C5 fragments. Complement activation was significantly down regulated in all three pathways for C5-depleted serum to which C5, preincubated with EspP, was added (whereas C5 preincubated with an EspP mutant was able to fully reconstitute complement activation). This indicates that EspP markedly destroyed the functional activity, as measured by a commercial total complement ELISA (Wieslab). Down regulation of complement by EspP in vivo may influence colonisation of EHEC bacteria in the gut or disease severity of HUS.

## 27. Increase of Extended-spectrum Beta-lactamase (ESBL) Producing Escherichia Coli in the Tyrol - a Matter of Clonality?

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**Introduction:** Beta-lactamases represent a major defence mechanism of gram-negative bacteria against beta-lactamase antibiotics. With the development of beta lactamase resistant antibiotics bacteria developed new mechanisms to overcome these new agents and responded with the production of extended-spectrum beta-lactamases (ESBLs). ESBL producing E.coli are increasing dramatically nowadays and have become a public health concern due to limited antibiotic options.
The aim of our study was to characterize ESBL producing E. coli in the Tyrol by investigating clonality via two different molecular typing methods and by determining the beta-lactamase profile.
**Methods:** One hundred twenty two ESBL producing E.coli, mostly from urine samples (n=97), from patients of the University hospital Innsbruck (n=60), district hospitals (n=20) and practitioners (n=41) in the Tyrol (Austria) were collected from January to April 2009.
Molecular comparison of the ESBL producing E.coli strains was done by repetitive PCR method (repPCR) (DiversiLab™) and Pulsed-Field Gel-Electrophoresis (PFGE). Serotyping of representative strains was performed according to the method of Ørskov & Ørskov. Characterisation of beta-lactamases was done by microarray analysis (Identibac®).
**Results:** Among 121 ESBL producing strains repPCR revealed one main clone comprising 99 isolates, twenty-two isolates showed different band patterns. PFGE corroborated these finding, but was even more discriminatory and could distinguish within the main clone, consisting of 90 of the 99 repPCR main clone isolates, between two subclones with subclone I consisting of 37 isolates and subclone II of 53 isolates. Serotyping of representative strains of both subclones showed, that they all belonged to the serotype O25:H4. Data of microarray revealed different combinations of beta-lactamases in ESBL producing strains of the main clone with TEM and CTX-M-type beta-lactamases being most prevalent.
**Conclusion:** ESBL producing E.coli in patients from hospitals and outpatients in the Tyrol show clonal distribution. Thus transmission of the same ESBL producing strain among patients might be the reason for the dramatic increase in this region in recent time.

## 28. The Role of Disturbed Innate Immunity Linked to Toll-Like Receptor 4 (TLR4) in Alpha-Synucleinopathies

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**Background:** The pathological hallmark of alpha-synucleinopathies are alpha-synuclein-positive cytoplasmic inclusions spread throughout the neurodegenerating central nervous system. Microgliosis commonly accompanies neurodegeneration in α-synucleinopathies and is considered a possible mediator of the disease process. Up-regulation of TLR4/CD14 has been reported in human MSA and PD/DLB brains as well as in murine transgenic models of neuronal and oligodendroglial alpha-synucleinopathy (Stefanova et al., 2007, Letiembre et al., 2007), however the role of innate immunity in these disorders remains unexplored. The current study addressed the role of TLR4 on microglial function in response to alpha-synuclein.

**Methods:** Immortalised murine microglial cell line BV-2 and primary murine microglia (P1-3) were used for all the experiments. The role of TLR4 was assessed by either using blocking antibodies, or preparing cell culture from TLR4 deficient mice. FACS analysis, immunocytochemistry and fluorescence microscopy were applied to characterise microglial activation and phagocytosis in response to fibrillar alpha-synuclein.

**Results:** Preliminary results suggest effects of α-synuclein on microglial activation and phagocytosis that is partly mediated through TLR4 and may be relevant to degeneration in alpha-synucleinopathies.

**Conclusion:** The current studies provide new insights into the role of innate immunity in alpha-synucleinopathies and define further pathogenic mechanisms that may play a role in neurodegeneration and identify potential novel therapeutic targets. This study is supported by grant of the Austrian Science Foundation P 19989-B05.

## 29. CYTIP is Targeted to Protein Degradation by SOCS1

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**Background:** CYTIP (Cytohesin interacting protein) is an intracellular molecule induced in dendritic cells (DC) during maturation. CYTIP, together with its binding partner cytohesin-1, accumulates at the contact zone between DCs and T-cells in co-cultures and modulates the binding potential of the adhesion molecule ICAM. If DCs are silenced for CYTIP, they keep longer contacts with T-cells, resulting in a lower activation in antigen-specific T cell stimulation assays. Thus, CYTIP plays a role in the de-attachment of T-cells from DCs, by interacting with its binding partner cytohesin-1. To further characterize the role of CYTIP in DC we searched for additional binding partners and found SOCS1 (Suppressor of cytokine signaling-1). SOCS1 negatively regulates the signaling of many cytokine receptors by inhibiting the JAK phosphorylation site of the receptors and by targeting JAK proteins for proteasomal degradation. Therefore we hypothesized that the interaction of CYTIP with SOCS 1 might limit the action of CYTIP through proteasomal degradation.

**Methods:** To verify a possible role for SOCS1 in taking CYTIP to the degradation machinery of the cell we measured endogenous CYTIP protein levels in mature DCs transfected with SOCS1 plasmid in different concentrations and at different time points in quantitative Western blot analyses.

**Results:** We observed lower amounts of endogenous CYTIP in mature DCs transfected with SOCS1 plasmid compared with untransfected DCs. These findings are most prominent 16h after transfection. To proof our findings, we plan to use a proteasome inhibitor (Bortezumib/VelcadeR) and repeat DC transfection with increasing amounts of SOCS1 plasmid. If protein levels of CYTIP remain unchanged, this would indicate that CYTIP binding to SOCS1 induces its degradation by the proteasome proving the hypothesis described above.

**Conclusions:** The findings described above indicate that DC, which use CYTIP to down-modulate the adhesion strength to T cells, further regulate this interaction by disposing of the molecule at later time points. This is in agreement with our view that CYTIP is used in the screening process of T cells, when MHC-peptide complexes are matched with specific T cell receptors for antigen. Once an antigen-specific interaction is formed, quick de-attachment of not matching cells and thus, CYTIP, is not needed anymore and is disposed of by proteasomal degradation.

## 30. The SiLISA® - a Diagnostic and Prognostic Biomarker-based Tool for Silicone-induced Fibrosis

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Silicone is the most widely used inorganic biomaterial in medical practice implemented in a large variety of active (cardiac pacemakers, cochlea implants, etc) and passive (drainage tubings, silicone mammary implants (SMis) etc.) implants. Although for decades silicone implants have been presumed to be biologically inert, they induce local inflammatory responses with fibrotic consequences (1). It also has been reported that silicone implants may be associated with an increased incidence of autoimmune disorders.

In the peri- SMI connective tissue capsule, we found massive infiltrates of immune cells, predominantly T-cells and macrophages, but also dendritic cells. Moreover, these various local and systemic side effects are associated with the proteinaceous film deposited on the surface of silicone implants, considered to be a key player in the activation of the innate and adaptive host defense mechanisms by promoting exposure of cryptic and/or altered self epitopes.

Based on this “protein signature” adhering to the surfaces of SMIs, we have invented a test system for the simple and simultaneous detection of eight of the major candidate proteins in patients’ sera using a modified ELISA test system (SiLISA®) (2,3).

The present work deals with the validation and improvement of the SiLISA® identifying the risk for developing fibrotic side effects to SMIs. With data from more than 100 subjects, we could successfully discriminate SMI- patients with a risk for fibrotic reactions to silicone compared to controls without complications.

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## 31. Pharmacokinetics of Intravenous Linezolid in Cerebrospinal Fluid and Plasma in Neurointensive Care Patients with Staphylococcal Ventriculitis Associated with External Ventricular Drains

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**Background:** Gram-positive catheter-related infection of the cerebrospinal fluid (CSF) pathway is a potentially life-threatening complication of external ventricular drainage (EVD). The aim of our study was to determine the pharmacokinetic profile of linezolid in CSF in patients with secondary obstructive hydrocephalus and staphylococcal EVD-associated ventriculitis.

**Methods:** Five patients requiring neurointensive care support received 600 mg of intravenous linezolid twice daily for 7 days. Concentrations of linezolid in CSF and plasma were analyzed with a validated high-performance liquid chromatography assay after single and multiple-dose administration. Pharmacokinetic parameters were estimated using standard noncompartmental methods. All patients completed the study without encountering severe adverse reactions.

**Results:** At steady state mean ± SD linezolid peak and through concentrations were 19.51 ± 5.1 mg/L and 1.94 ± 1.69 mg/L in plasma and 7.11 ± 2.20 mg/L and 3.09 ± 1.75 mg/L in CSF, respectively. Mean ± SD area under concentration-time curve at steady state was 86.54 ± 44.54 mgh/L for plasma and 63.02 ± 18.93 mgh/L for CSF with a CSF to plasma ratio of 0,79 ± 0.25. At steady state the times above MIC in CSF were 99.8% and 57.2% for pathogens with MIC values of 2 mg/L and 4 mg/L, respectively, and resulted in microbiologically and laboratory-confirmed CSF clearance and clinically favorable outcome.

**Conclusions:** The present study indicates that the intravenous administration of linezolid 600 mg twice daily to patients with acute neurological illness requiring critical care support should provide sufficient antimicrobial concentrations in the CSF exceeding the susceptibility breakpoints for most gram-positive bacteria. Linezolid application can be considered a safe and efficacious treatment of drain-associated ventriculitis caused by susceptible pathogens.

## 32. T Regulatory Cells and TH17 Cells in Peri-Silicone Implant Capsular Fibrosis

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In this study, we investigated the immunological mechanisms underlying the extensive peri-silicone implant capsule formation, one of the most frequent post-operative complications in patients receiving silicone mammary implants (SMI). We studied immune response activation by phenotypic and functional characterization of lymphocytes accumulated within the peri-implant fibrotic tissue capsule. Intracapsular lymphoid cells and autologous peripheral blood mononuclear cells (PBMCs) were isolated and analyzed via flow cytometry. The expression of T regulatory cells ($CD4^+CD25highFoxp3^+CD127^-$) (Tregs), cytokine profiles, and the T-cell-receptor (TCR) repertoire of these cells were examined. Intracapsular Tregs were further analyzed by immunohistochemistry and functional suppression assays. In comparison to peripheral blood, the cellular composition of intracapsular lymphocytes showed a predominance of $CD4^+$ cells with a significantly increased number of TCR gamma/$delta^+$ cells. Intracapsular T cells predominantly produced interleukin (IL)-17, IL-6, IL-8, transforming growth factor (TGF)-beta1, and interferon (IFN)-gamma, suggesting a TH1/TH17 weighted local immune response. In addition, intracapsular T-cells displayed a restricted TCR alpha/beta repertoire. The suppressive potential of Tregs was demonstrated in autologous mixed lymphocyte reaction with peripheral T cells; however, they did not suppress intracapsular T cells. Interestingly, ratios of intracapsular Tregs were inversely proportional to the clinical degree of capsular fibrosis. Our results indicate that silicone implants trigger a specific, antigen-driven local immune response through activated TH1/TH17 cells suggesting that fibrosis is promoted by the production of profibrotic cytokines and controlled by the local Tregs.

## 33. Additive Roles of the Protein Kinase C θ and PKC α Isotypes in T-cell Immune Responses in vivo

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**Background:** The purpose of the study is to investigate the potential overlapping roles of the two distinct Protein Kinase C (PKC) family members PKC α and PKC θ in T cell activation processes.

**Methods:** The established role of PKC θ in T cell dependent immune responses has focused extensive efforts towards developing immunosuppressive therapeutics targeting this PKC isotype. Therefore, a complete understanding of the physiological roles of all other PKC isotypes is required. PKC θ is known to be a key player in the T cell signaling cascade [PFEIFHOFER et al., 2003 J Exp Med; SUN et al., 2000 Nature]. We defined PKC α as the PKC isotype that is necessary for T cell dependent IFN-γ production and IgG2a/2b antibody responses in vivo [PFEIFHOFER et al., 2006 J Immunol]. Thus, we generated, additionally to our PKC α and PKC θ single knockout mice (KO), PKC α/θ double knockout (DKO) mice in order to explore a possible additive effect in T cell signaling.

**Results:** In vivo transplantation experiments demonstrated that heart allograft survival was significantly prolonged in PKC α/θ DKO recipient mice. Anti-CD3 antibody injection induced an impaired interleukin-2 (IL-2) response, which was stronger reduced in the PKC α/θ DKO mice. Furthermore, PKC α/θ DKO showed an additively reduced nuclear translocation and DNA binding capacity of NFAT.

**Conclusions:** PKC α and PKC θ functionally cooperate to upregulate NFAT translocation and DNA binding, thereby activating effector genes as IL-2 to subsequently trigger T cell responses in vivo. Consistently, gene ablation of both PKC α and PKC θ leads to a synergistic elongation of heart allograft survival rates. The reduced IL-2 plasma levels are most likely responsible for the strong immunosuppression phenotype in the PKC α/θ DKO mice. Thus, the combined inhibition of PKC α and PKC θ may serve as an innovative combinatorial drug treatment concept in order to inhibit T cell immune functions in clinical immunosuppression regimes in vivo.

## 34. Divergent Modulation of Chlamydia Pneumoniae Infection Cycle in Human Monocytic and Endothelial Cells by Iron, Tryptophan Availability and Interferon Gamma

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**Background:** Chlamydia pneumoniae is an obligatory intracellular bacterium causing chronic inflammatory diseases in humans. We studied the role of the nutritive factors, iron and tryptophan, towards the course of infection and immune response pathways in C. pneumoniae infected endothelial cells and monocytes.

**Methods:** Human endothelial (EA.hy923) and monocytic cells (THP-1) were infected with C. pneumoniae, supplemented with iron or 1-Methyltryptophan (1-MT), an inhibitor of the tryptophan degrading enzyme indoleamine 2,3-dioxygenase (IDO), and subsequently stimulated with IFN-γ or left untreated. The number of infected cells, the morphology and quantity of C. pneumoniae inclusion bodies, IDO activity and innate immune effector pathways were analysed.

**Results:** While neither iron challenge, IDO inhibition or IFN-γ treatment had a significant effect on C. pneumoniae morphology or numbers within THP-1 monocytic cells, iron supplementation to EA.hy926 cells resulted in promotion of C. pneumoniae proliferation and differentiation while IFN-γ had an inhibitory effect. Furthermore, the number of infected endothelial cells was significantly decreased upon 1-MT treatment. C. pneumoniae infection induced a pro-inflammatory immune response as evidenced by increased IDO activity, neopterin formation or TNF-α production in THP-1 but not in endothelial cells. These pathways were superinduced upon IFN-γ treatment and partly modulated by iron supplementation.

**Conclusion:** Our results demonstrate that the infectious cycle of C. pneumoniae behaves differently between monocytic and endothelial cells. While the intracellular pathogen remains in a persistent form within monocytes, it can differentiate and proliferate within endothelial cells indicating that endothelial cells are a preferred environment for Chlamydia. Nutritive factors such as iron have subtle effects on C. pneumoniae biology in endothelial, but not monocytic cells. Our results contribute to a better understanding of C. pneumoniae infection and its role for chronic inflammatory diseases such as atherosclerosis.

## 35. The Inflammatory Response in Atopic Dermatitis

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Currently, atopic dermatitis (AD) is seen as a result of combined altered barrier function, abnormal immune reactivity and environmental factors such as allergens and microbes. Impaired epidermal barrier function can be due to inherited epidermal abnormalities such as mutations in the gene encoding for the epidermal protein filaggrin. Vitamin D3-induced overexpression of thymic stromal lymphopoietin (TSLP) by keratinocytes results in an AD-like inflammatory phenotype in mice echoing the discovery of high TSLP expression in epidermis from AD patients. We here report that in a mouse model of AD involving abnormal skin immune reactivity i. e. mice treated with the low-calcemic vitamin D3 analogue, MC903, AD-like inflammation and symptoms depend on the presence of epidermal Langerhans cells (LC). Accordingly, expression of maturation markers by LC is increased whereas maturation of dermal DC is not altered. Moreover, only LC are responsible for the polarization of naive $CD4^+$ T cells to a Th2 phenotype i. e., decrease in IFN-$\gamma$ and increase in IL-13 production by $CD4^+$ T cells. Moreover, mice deficient for filaggrin which exhibit inherited abnormal epidermal barrier function show enhanced inflammatory dermal infiltrates and plasma IgE levels compared to control mice when repeatedly treated with oxazolone to induce AD-like symptoms. In a mouse model that combines abnormal skin immune reactivity and epidermal barrier function i. e, mice topically treated with vitamin D3 after tape stripping, we show that tape-stripping does not worsen the AD-like symptoms induced by vitamin D3 treatment. Indeed, abnormal epidermal barrier function alone does not induce major skin inflammatory response in contrast to vitamin D3 treatment which increases plasma IgE levels, dermal inflammatory infiltrates, and emigration of LC. Interestingly, patients with a filaggrin deficiency associated with atopic ichthyosis vulgaris have enhanced LC density in the epidermis independent to the presence of eczema. Moreover, their LC exhibit an activated morphology. In contrast, patients with a filaggrin deficiency associated with ichthyosis vulgaris without atopic symptoms have normal LC density. Therefore, atopic symptoms are tightly linked to a modification of the number and the phenotype of LC.

## 36. Targeting a Kv1.3 Potassium Channel for Immunosupression in Reconstructive Transplantation

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**Background:** Skin rejection in reconstructive transplantation is primarily driven by a T-lymphocyte driven immune response towards the epidermis. Kv1.3 potassium channels on lymphocytes are critically involved in T cell activation. The effect of correolide C, a blocker of Kv1.3 was investigated in a rat limb transplant model.

**Methods:** After orthotopic rat hind limb allotransplantation (BN-LEW) animals received correolide C either i.p. (5mg/kg/day) or as intra-graft treatment (3mg/kg twice/week s.c. into the limb) in combination with tarolimus, given i.p. for 30 days (0.3mg/kg/day) or 50 days (0.3mg/kg/day0-30 and 0.1mg/kg/day31-50). Untreated animals, placebo treated animals and animals receiving tacrolimus alone served as controls. Rejection was assessed by daily inspection and H&E-histology of skin biopsies. Grade III rejection was defined as end-point. Tacrolimus 24h-trough blood levels were measured regularly after pod 30. WBC and RBC counts were recorded in native and correolide C (i.p. only) treated animals.

**Results:** Untreated and placebo treated controls rejected at day 8.83+/-0.98 and 9.00+/-2.83 (p=0.894). When given i.p., correolide C monotherapy resulted in slight but significant prolongation of allograft survival (10.50+/-1.38, p=0.037). Histology showed only a mild lymphocytic infiltrate and single vacuolized keratinocytes in the epidermis on pod 10 in 4/6 correolide C treated animals. RBC counts were decreased, whereas WBC counts were increased in correolide treated animals on pod 14, compared to native animals (RBC: 4.80+/-1.07 vs. 8.44+/-0.58, p=0.00023; WBC: 30.74+/-1.40 vs. 13.20+/-3.27, p=0.000097). After weaning tacrolimus on pod 30, limbs were rejected by pod 40.00+/-1.00 (grade III), and histology revealed necrosis of the epidermis. Additional treatment with local correolide C resulted in an insignificant prolongation of graft survival (pod 43.00+/-3.74; p=0.24). 2/5 animals showed intact skin with a mild dermal infiltrate until day 45. Weaning tarcolimus on pod 50 resulted in rejection of the limb by day 55.00+/-0.00 regardless of correolide therapy. Tacrolimus mean blood levels were 2.97+/-0.98 ng/ml when tacrolimus was given at 0.3mg/kg/day and undetectable (<0.6 ng/ml) 5 days after weaning.

**Conclusions:** Systemic administration of a Kv1.3 blocker results in slight prolongation of graft survival after rat hind-limb allotransplantation while local administration into the skin has no effect under low dose tacrolimus therapy.

## 37. Secretable Antiviral Entry Inhibitory (SAVE) Peptides for Gene Therapy of HIV Infection

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**Background:** C peptides (e.g. T20, C46) are highly efficient inhibitors of HIV entry. Secreted from gene-modified cells, C peptides are expected to mediate a bystander protective effect on neighboring non-modified cells and suppress virus replication even if only a small fraction of cells is genetically modified.

**Methods:** Short peptides are only inefficiently translated and exported by the cellular secretory machinery. To circumvent these limitations we expressed therapeutic C peptides as concatamers which were subsequently processed into monomeric peptides by protease cleavage within the secretory pathway.

**Results:** Transfection or transduction of cell lines with retroviral vectors resulted in high-level expression and secretion of SAVE C peptides, which exerted a high antiviral activity in single-round infection assays with replication incompetent lentiviral particles pseudotyped with a variety of different HIV envelope glycoproteins. Moreover, genetic modification of primary human T cells resulted in the secretion of therapeutic levels of C peptides. In mixed cell cultures SAVE peptides secreted from transduced cells produced a bystander effect and suppressed HIV-1 infection of non-modified cells.

Currently, efficacy of the secreted antiviral peptides is analyzed in humanized mouse models of HIV infection using lentiviral and Adeno-associated Virus (AAV) vectors for SAVE peptide expression.

**Conclusion:** The in vivo secretion of therapeutic C peptides from gene-modified T cells holds great promise as the cells would be expected to home to lymphatic tissues, which are the major sites of HIV replication. Secretion of the antiviral gene product in the lymphatic tissues is likely to lead to high and stable local concentrations and confer a substantial antiviral effect.

## 38. Th17 / Th1 Biased Naturally Acquired Immunity to the Pneumococcal Proteins PcsB, StkP and PsaA in Adults of Different Age

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**Background:** Streptococcus pneumoniae is one of the major human pathogens, causing high morbidity and mortality especially in children and in the elderly, which are particularly susceptible to *S. pneumoniae* infections due to the dysregulated function of the aged immune system. As the present generation of poylsaccharide vaccines do not give sufficient protection in adults, new vaccination strategies are urgently needed.

**Methods:** To test, whether pneumococcal proteins were able to induce adaptive immune responses in adults of different age, we investigated serum IgG antibody titers and T cell immunity (IFN-$\gamma$, IL-17A and IL-5 production) to the three pneumococcal proteins, PcsB, StkP and PsaA, in 112 healthy adults of three different age groups (young, middle aged and elderly).

**Results:** While more than 70 percent of young and middle-aged persons had IgG against one or more pneumococcal proteins, a significantly lower percentage of elderly persons were IgG positive. This was due to the fact, that in old age antibody responses to PcsB were rare. In all age groups, a lower percentage of donors had antibodies against PsaA (< 20 %) than against the two other proteins. There was also no age-related difference in the number of persons who had a cellular immune response against one or more pneumococcal proteins. Again, PsaA was less frequently recognised than the other two proteins. A Th17 response was predominant in all age groups and was combined with a Th1 response in young and middle aged but rarely in elderly persons. A combination of both, a humoral as well as a cellular immune response was observed in the majority of young and middle aged persons but hardly in the elderly group. Elderly persons still frequently had a cellular response in the absence of antibodies.

**Conclusion:** The results demonstrate that in the majority of adults there is a naturally acquired immune response to pneumococcal proteins. Absence of antibodies can be compensated by a Th17 dominated cellular response in old age.

## 39. An Experimental Study of the Use of Bioabsorbable Poly (l-lactic acid) / Poly (ethylene oxide) Microspheres Containing Vancomycin Chlorhydrate for Bone Repair

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**Background:** Bone grafts and/or synthetic bone substitutes are commonly used to repair tissue loss due to injury. In these processes, antibiotic therapy is necessary to prevent and combat bacterial agents that could eventually delay the tissue repair.

**Methods:** The present study evaluated an antibiotic in combination with bioabsorbable polymeric implants for bone tissue. Poly (L-lactic acid) / poly (ethylene oxide) microspheres were implanted in rats and evaluated for periods of 2 days until 32 weeks. Groups implanted with antibiotic loaded and non-loaded blends were compared.

**Results:** Fibrin net and hemorrhage areas were observed primarily around the microspheres which were replaced by granulation tissue. Woven bone formation with progressive maturation was observed.

**Conclusions:** Mixing of vancomycin chlorhydrate into polymer microspheres did not affect bone regeneration. The material could be used as bone graft, improving bone repair. Antibiotic association could be useful to prevent infections during bone healing.

## 40. Use of Poly (L-lactic acid) PLLA as Bone Grafts Substitute after Benign Tumor Resection. A Preliminary Clinical Trial

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**Background:** Bone grafts are often used as bone defects repair resulting from trauma, infectious diseases or bone tumors resections. Limitations are associated with the possible inflammation processes at the donor site, besides the limitation in the quantity and shape of the obtained grafts.

**Methods:** In this present study a bioreabsorbable and biocompatible polymer was used as bone grafts substitute. For that the case of four patients after the removal of benign tumors from different areas was followed. After the removal of benign tumor material, the cavities were filled with grains of poly (L-lactic acid) PLLA.

**Results:** After one year, X-rays shows successful bone healing and tissue integration with PLLA grains.

**Conclusions:** The present study shows that PLLA is an option for the treatment of small bone defects.

## 41. Impact of Life Span on Treg Cell Maturation and Function

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Both apoptosis and regulatory T cells (Treg cells) are key players in maintenance of immunological homeostasis and tolerance. To investigate how survival factors impact on Treg cell maturation and function we crossed foxp3-gfp reporter mice with bim-/- and vav-bcl-2 transgenic mice allowing easy isolation of highly pure Treg cells by cell sorting. Subsequently, we analysed their apoptosis susceptibility to different death stimuli, their abundance and phenotype in vivo as well as their suppression capacity in an in vitro suppression assay. We could show that $CD4^{+}FoxP3GFP^{+}$ Treg cells compared to $CD4^{+}FoxP3GFP^{-}$ conventional T cells are more susceptible to cell death induced by cytokine withdrawal, histone deacetylase inhibitors and Fas-FasL interaction. In contrast they are more resistant to apoptosis mediated by glucocorticoids, the DNA damaging drug etoposide and the kinase inhibitor staurosporine. Although the percentage and absolute number of Treg cells was increased in bim-/- and vav-bcl-2 transgenic mice expression of typical Treg cell markers (FoxP3, CD25, GITR, CTLA-4) was reduced. Furthermore, this was accompanied by a reduced suppression capacity of bim-/- and vav-bcl-2 Treg cells. To transfer our in vitro results to in vivo conditions we are now assessing the suppressive function of bim-/- and vav-bcl-2 Treg cells in a murine autoimmune model of colitis.

## 42. Outcome of Liver Transplantation in Recipients Older than 65 Years: A single center experience

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Advanced age was considered a relative contraindication for liver transplantation (LT) in the past. The percentage of older patients referred for LT assessment has markedly increased in almost all LT centers over the last years. The data regarding the outcome of older patients have been contradictory. Therefore, we aimed to evaluate the long-term survival of LT recipients older than 65 years.

Between 1982 and 2008, 106 out of 1011 patients (10.5%), who underwent LT at our institution, were older than 65 years. Almost all of these patients were transplanted after 1995. The mean age was 67.5 (65.0 – 76.4) years and the majority of patients were male (76%). Regarding underlying liver disease the percentage of non-alcoholic steatohepatitis associated cirrhosis was significantly higher in the older age group (15.9% vs. 5.8%, $p< 0.01$) compared to the younger LT cohort. In contrast, alcoholic liver disease was more common in recipients under 65 years (20.4% vs. 14.1%, $p < 0.01$). Significantly more patients in the older group presented with a concomitant hepatocellular carcinoma (40.6% vs. 23.7%, $p < 0.01$). Both groups did not differ with regard to Child-Pugh classification and mean MELD score. The median follow-up of the older patients was 3.2 compared to 5.7 years of the younger cohort.

The actuarial patient survival rates at 1-, 5- and 10-years with 85%, 65% and 56% were slightly lower in the older recipients compared to 87%, 75% and 66% of recipients ≤ 65 years. The major causes of death in the elderly were sepsis (n=13) in the early postoperative period and de-novo cancer (n=6), HCV/HCC recurrence (each n=5) and cardiovascular complications (n=4) in the late one. The incidence of de-novo cancers and cardiovascular diseases were significantly higher in the older age cohort. The percentage of reLTs did not differ between both groups (7.8% vs. 6.5%).

Although overall survival was better for younger patients, this study shows that LT recipients older than 65 years have a favourable outcome after LT with a 5- and 10-year survival of 65% and 56% indicating that liver transplantation should be considered in patients older than 65 years.

## 43. Viral Load Predicts Outcome of Hepatitis C Patients after Liver Transplantation

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Recurrent hepatitis C (HCV) infection is almost ubiquitous after liver transplantation (LT). Risk factors associated with HCV recurrence include donor, recipient and viral parameters. Conflicting data have been reported regarding viral load and severity of recurrent HCV disease. The aim of this study was to analyse the impact of viral load within the first year after LT on severity of recurrent HCV infection.

Between 1980 and 2006 175 patients were transplanted due to HCV-cirrhosis at our institution. Only patients (n=128, age: 56.8±8.9 years; 31 females, 99 males) who survived more than 6 months and with histological assessment of recurrent HCV infection were included in this study. Non of the excluded patients died due to HCV recurrence. Viral loads were measured at week 2, month 3, 6 and 12 post LT, using the bDNA HCV RNA 3.0 assay (Bayer Diagnostics). 34 patients (18.4%) developed either a cholestatic type of HCV recurrence (n=16; 8.6%) and / or a rapid progression to advanced fibrosis / cirrhosis (n=23, 12.4%). The overall follow-up was 6.1 years.

The actuarial patient survival of all patients at 1-, 5- and 10 years were 87%, 75% and 59%. Patients with cholestatic type recurrence and advanced fibrosis had significantly decreased survival rates at 1-, 5- and 10 years with 90%, 65% and 45%, compared to patients with mild/moderate or no recurrent disease with 98%, 81% and 71%. Cox regression analysis showed that the development of a cholestatic recurrence, patients' age and viral load at week 2 were associated with poor patient survival. Although higher viral loads were seen in patients with a severe recurrence at any time, multivariate binary regression analysis showed that only viremia at week 2 and donor age were predictive factors for the cholestatic HCV recurrence in contrast to viral loads at months 3 and 6 together with recipient age and cold ischemic time for the rapid development to advanced fibrosis. Genotype, immunosuppression and several other parameters did not reach statistical significance.

Our study shows that viral load is an important parameter for the development of severe recurrent disease and recipient survival after LT. Whereas early viremia is highly predictive for the cholestatic type, high viral loads between month 3 and 6 are associated with advanced fibrosis/cirrhosis of the liver allograft.

## 44. Outcome of Patients with Recurrent Hepatocellular Carcinoma after Liver Transplantation

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Although current selection criteria have led to a significant decrease in HCC recurrence rates after liver transplantation (LT), recent studies have reported recurrent rates around 10%. So far, only few studies have addressed therapeutic options and outcome of patients with recurrent HCC. Therefore, the aim of our study was to evaluate the survival of patients with HCC recurrence and to identify specific risk factors.

We analyzed the data of 202 HCC patients (26 f/176 m). Their median age was 60 (31–75) years. The main underlying liver diseases were viral cirrhosis in 51% and (non) alcoholic fatty liver disease in 33%; 40% had CPC stage A, 46% B and 14% C. PreLT TACE was performed in 138 patients, RFA in 8 and a combination of both in 10 cases. The mean follow-up was 6.1 years.

The actuarial patient survival rates at 1-, 5- and 10-years were 85%, 65% and 55%. HCC recurrence was found in 36 patients (18%) after a median time of 1.2 (0.2 – 7.4) years after LT. Patients survival at 5 and 10 years was considerably lower for patients with recurrent disease (27%, 0%) than without (73%, 68%). Recurrence was limited to the liver or lymph nodes in 6 and 4 patients, 26 patients presented with metastatic disease. Twenty-four (67%) patients died due to recurrent HCC leading to a median survival after recurrence of 0.8 (0.1 – 5.5) years. The outcome of patients with recurrent HCC was significantly better if surgical or local ablative treatment options (n=9) were possible compared to systemic chemotherapy (n=9) or best supportive care (n=18) with median survival rates of 42.6 vs. 11.9 vs. 4.2 months (p=0.005). In multivariate analysis preLT therapy did not influence HCC recurrence, but complete response to pre LT treatment was associated with a significantly lower risk of recurrence compared to partial or no response. Recurrence correlated significantly with poorly differentiated tumors, vascular invasion, advanced UICC stage and tumor size.

Patients suitable for surgical or loco ablative therapy have an excellent prognosis despite recurrent HCC. This warrants an intensive post-transplant surveillance program to diagnose HCC recurrence at an early stage.

## 45. Liver Transplantation for Patients with Acute on Chronic Liver Failure: An intention to Treat Analysis

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Currently the CLIF (chronic liver failure) consortium was endorsed by the European Association for the Study of the Liver (EASL) in order to stimulate research in the field of chronic liver failure. Our center was invited to join this international research group. For this reason we retrospectively analyzed our date regarding patients with acute on chronic liver failure (ACLF), defined as acute deterioration of a patient with compensated liver disease. ACLF carries a high risk of mortality mainly due to multi-organ dysfunction. Liver transplantation (LT) is the only curative therapeutic option for these patients. The aim of this study was to determine the outcome of patients with ACLF and, in particular, the efficacy of LT in the treatment of these patients referred to our tertiary center.

Eighty-four consecutive patients with decompensated liver cirrhosis who required intensive monitoring and/or could not be treated outside an intermediate care unit were included in this study. The mean age was 55 years (43% female, 63% male). The major underlying liver diseases were (non) alcoholic fatty liver disease (50%) and viral cirrhosis (27.4%). At admission the mean MELD, MELD Na and SOFA score were 26.6, 29.6 and 9.4. Infections, in particular SBP and pneumonia, and acute alcoholic hepatitis were the major precipitating events for decompensation.

Based on an intention to treat analysis the median overall survival was 1.58 (95%CI: 0.84-2.32) months. Sixty (71.4%) patients were evaluated, but only 45 (53.6%) were actually listed for LT. Severe sepsis and acute alcoholic hepatitis were the main reasons not to consider LT. Out of the 45 patients listed for LT, only 15 could be successfully transplanted. The remaining patients died on the waiting list mainly due to sepsis and multi-organ failure leading to a waiting list mortality rate of 67%. In the post-operative period only two patients died within the first year after LT because of sepsis and graft versus host disease. The other patients had an uneventful post LT course leading to a 1- and 5-year survival rate of 85%. In multivariate analysis LT was the only positive predictive parameter, whereas sepsis, HRS 1, intubation and multi-organ failure were negative predictive factors for patient survival.

Our study confirms the poor prognosis of patients with ACLF. Although more than 70% were evaluated for LT, only 18% could be successfully transplanted. Infectious complications were the main reasons for a refusal of LT and/or death on the waiting list. The postoperative outcome, however, was excellent with a 5-year survival rate of 85%.

## 46. Homeostasis of Inducible Regulatory T-cells (iTreg)

G. J. Wiegers, D. Tischner, A. Villunger

It is now recognized that regulatory T-cells (Treg) that express the transcription factor FoxP3 play a key role in the maintainance of immune homeostasis and prevention of autoimmune diseases. $FoxP3^+$ Treg cells can be divided into two types: natural Treg (nTreg) cells, that develop in the thymus, and adaptive (or induced) Treg (iTreg) cells that are generated outside the thymus in the presence of TGF-beta and other factors. It is thought that iTreg cells are important for mucosal immune tolerance and control of chronic allergic inflammation. We investigated how factors known to affect life span impact on (the function of) iTreg cells and compared the results to both conventional T-cells (Tcon) that do not express FoxP3, and to nTreg cells. Both in vitro generated iTreg cells and nTreg cells appeared more sensitive to cell death induced by cytokine withdrawal or histone deacetylase inhibitors than Tcon cells. In contrast, both types of Treg cells are less sensitive to the kinase inhibitor staurosporine than Tcon cells. Interestingly, while nTreg cells were more susceptible to Fas-FasL induced apoptosis than Tcon cells, iTreg cells appeared resistant to this form of apoptosis. Moreover, iTreg cells were resistant to anti-CD3-induced death (i.e. activation-induced cell death (AICD)) that is known to be mediated by Fas-FasL interaction. Surprisingly, anti-CD3 rescued iTreg cells from spontaneous cell death, even in the absence of IL-2. To explain these findings, we analyzed Fas expression by iTreg cells and found reduced levels compared to Tcon cells. Finally, TGF-beta plays a crucial role in that it rescued Tcon cells from AICD but reversed the rescue of iTreg cells by anti-CD3. We are currently investigating which proteins are modulated by TGF-beta to explain these cell type specific findings.

## 47. Deletion of the Signal Transduction Molecule p14 under the CD11c Promotor Impairs the Development of Murine Langerhans Cell Network

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**Background:** Dendritic cells (DC) are important regulators of immunity and tolerance. To fullfill their antigen presenting capacity, DC need to process and distribute incorporated antigen via endosomal sorting to distinct cellular compartments so that they can present it to effector T cells. The extracellular signaling-regulated kinase (ERK) cascade is involved in endosomal sorting processes. Hence, we investigated the role of the adaptor molecule p14, an essential part of the ERK cascade, in the context of DC function.

**Methods:** We generated a DC specific knock out mouse model by Cre-CD11c-mediated ablation of p14. Phenotypical analysis of the DC populations was carried out by flow cytometry analysis as well as with immunofluorescence microscopy of epidermal sheets and cryostat sections.

**Results:** The overall numbers of $CD11c^+$ DC in spleens and lymph nodes were increased in knock-out compared to wildtype mice. However, within the fraction of highly MHC II expressing DC in the skin-draining lymph nodes we noted greatly diminished numbers of both langerin+/CD103-negative (migrated "genuine" Langerhans cells) and $langerin^+/CD103^{-+}$ cells (migrated $langerin^+$ dermal DC). The reduced number of skin DC, especially epidermal Langerhans cells was further confirmed by quantitative and qualitative analysis of the skin of the mice. Investigating the ontogeny of Langerhans cells by analysing the skin of newborn mice, revealed, that Langerhans cells are capable of establishing their epidermal network within 3 days after birth. However, the maintainance and homeostasis of the network seems to be affected in p14 knock-out mice as indicated by a constant loss of Langerhans cells starting around day 6 after birth.

**Conclusion:** In summary, our observations identify p14 as an important molecule regulating the homeostasis of the Langerhans cells network. The molecular basis for this phenomenon is currently being investigated.

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## 48. IL15 DCs Loaded with Complement-opsonised HIV Efficiently Induce Expansion of Naive CD8+ T cells

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**Background:** Recent in vitro experiments revealed differences with respect to productive infection of immature IL4-generated DCs with differentially opsonised HIV. Furthermore, the antigen-presenting capacity of these cells differed dependent on the opsonisation pattern of the virus. Since IL15 DCs were demonstrated to efficiently prime naive $CD8^+$ T cells to differentiate into melanoma-specific CTLs, we tested if IL15 DCs loaded with differentially opsonised HIV are more potent in stimulating $CD8^+$ T cell expansion.

**Methods:** First, we characterised IL15 and IL4 DCs after exposure to LPS or differentially opsonised HIV, for DC markers, complement receptors and FcgammaRs by FACS analyses or RT-PCR. Subsequently, IL4 and IL15 DCs were infected with differentially opsonised R5-, R5X4- and X4-tropic HIV preparations and virus production was monitored over several days after infection by p24-ELISA. Finally, we investigated whether HIV-loaded IL15 DCs are more potent in initiating expansion of autologous naive $CD8^+$ T cells compared to HIV-IL4 DCs.

**Results:** We found that similar to IL4 DCs, all HIV preparations activated IL15 DCs with regard to characteristic maturation markers. As observed with IL4 DCs, infection of IL15 DCs was enhanced when HIV was coated with complement fragments compared to non-opsonised virus (HIV) or HIV opsonised with specific IgGs (HIV-Ig). However, IL15-DCs exposed to HIV-C were more efficient in inducing proliferation of naive $CD8^+$ T cells than IL4 DCs loaded with HIV-C. The specificity and functionality of the in vitro generated $CD8^+$ T cells were furthermore verified by CD107a mobilization experiments, tetramer staining and testing the anti-viral activity against HIV-1 infected autologous $CD4^+$ T cells.

**Conclusion:** Our results indicate that IL15 DCs exposed to complement-opsonised HIV are superior in inducing expansion and differentiation of HIV-specific CTLs compared to HIV-C-IL4 DCs.

## 49. Langerhans Cells and Dermal Langerin$^+$ Dendritic Cells Transport Antibodies Targeting the C-type Lectin DEC-205 in vivo, but are not Essential to Subsequent Cytotoxic Responses

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Antigens deposited in the skin, such as vaccines given subcutaneously, are captured by different cutaneous dendritic cells (DC), but also DC residing in secondary lymphoid organs.

We found that intradermal injection of mAb to C-type lectin receptors DEC-205/CD205 and langerin/CD207 resulted in strong and rapid labelling of epidermal Langerhans cells (LC); this implies diffusion of large molecules through the basement membrane into the epidermis. Anti-DEC-205 also targeted langerin$^+$/CD103$^+$ and langerin$^-$/CD103$^-$ dermal DC. Following in vivo uptake of ovalbumin-coupled anti-DEC-205, LC isolated by migration from epidermal sheets potently induced proliferation of ovalbumin-specific CD4$^+$ and CD8$^+$ T cells in vitro, suggesting that LC efficiently present receptor-targeted antigens administered in the skin.

In vivo, the targeted skin DC migrated through lymphatic vessels in steady state and inflammation. The transport of the targeting mAb to skin-draining lymph nodes was strongly dependent on migrating skin DC, most of which were langerin$^+$. Transport was increased upon topical skin treatment with the TLR7 agonist imiquimod. Participation of dermal langerin$^+$ DC transiently increased upon inflammation. Complete removal of the site where ovalbumin-coupled anti-DEC-205 had been injected substantially decreased endogenous cytotoxic responses against ovalbumin peptide-loaded target cells. Surprisingly, selective ablation of langerin$^+$ DC by means of langerin-diphtheria-toxin receptor knock-in mice did not affect such responses, independently of the adjuvant chosen (imiquimod, poly I:C).

Thus, in the context of cutaneous targeting of DC in vivo, langerin$^+$ skin DC play a major role in transport of DEC-205-bound mAb, but appear redundant for the induction of subsequent CD8$^+$ T cell responses.

## 50. Complement as an Endogenous Enhancer for Dendritic Cell-mediated Induction of HIV-specific CTLs

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**Background:** Previous studies have demonstrated the involvement of complement (C) in induction of efficient CTL responses against different viral infections, but the exact role of complement in this process has not been determined. We now show that C opsonization of retroviral particles enhances the ability of dendritic cells (DCs) to induce HIV-specific CTL responses in vitro.

**Methods:** In vitro prime-boost experiments of naive $CD8^+$ T cells with autologous, differently loaded DCs were performed. The DC-induced $CD8^+$ T cell expansion and functionality (IFN-$\gamma$ secretion, degranulation efficiency, tetramer recognition) were analyzed by FACS. Additionally, the antiviral efficiency of the in vitro generated CTLs was monitored by p24 ELISA.

**Results:** The prime-boost experiments revealed that DCs exposed to complement-opsonized HIV (HIV-C) mediated significantly higher $CD8^+$ T cell expansion and IFN-$\gamma$ secretion than DCs loaded with non-opsonized HIV (HIV). Additionally, HIV-C-DC-generated $CD8^+$ T cells were able to degranulate upon specific peptide stimulation, to recognize an HIV-gag-immunodominant epitope and to elicit antiviral activity significantly better than HIV-DC-CTLs.

**Conclusion:** Our results indicate that complement serves as natural adjuvant for DC-induced expansion and differentiation of HIV-specific CTLs.

## 51. Thrombocytes as Antifungal Effector Cells

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**Background:** Thrombocytes, traditionally known for their crucial role in haemostasis, are now recognized to fulfil important effector functions in the antifungal host defence. Human platelets are described to interact directly with Aspergillus. The consequences of this interaction are the expression of activation markers and a significant decrease of fungal galactomannan release, colony size and hyphal elongation. Our current project aims to further define the prerequisites and parameters for the interplay between fungus and thrombocytes. Furthermore, we investigate the influence of Aspergillus-derived mycotoxins on this process.

**Methods:** Thrombocytes were isolated from human blood and used either as platelet-rich plasma or after purification using a sepharose column. The incubation with Aspergillus fumigatus or A. terreus was performed either in presence or in absence of serum as complement source. Surface activation markers on the platelets such as CD62P and CD63 as well as generation of microparticles were quantified by FACS analysis. The exposure of phosphatidylserine was determined by staining with annexin V. These parameters were partly measured in the presence of the mycotoxins Gliotoxin and Patulin.

**Results:** Whereas former experiments with bacteria implicated that complement is necessary for an efficient antimicrobial reaction we could not prove a significant enhancement of platelet-fungus interaction by opsonization and activation of the complement pathways. Activation of thrombocytes after contact with Aspergillus terreus and A. fumigatus was clearly shown by upregulation of CD62P surface expression. Furthermore, staining of the cells by annexin V, a marker for both activation and apoptosis of thrombocytes, was significantly enhanced. FACS analysis indicated the appearance of microparticles as a consequence of incubation of platelets with the fungi. High concentrations of the mycotoxins Gliotoxin and Patulin were necessary to modify platelet activation.

**Conclusion:** The contact between human platelets and fungal pathogens results in a broad spectrum of activation steps and signal transduction mechanisms. Further experiments aim to define the corresponding receptor molecules. Mycotoxins seem to play a role in this process only in high concentrations.

## 52. Various Groups of Pathogenic Fungi Produce Proteases that can Degrade Immune Proteins in the CNS

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**Background:** Invasive fungal infections generally show a high lethality rate of more than 90% if the central nervous system (CNS) is affected. As the blood-brain-barrier for the most part prevents the entry of components of the peripheral host defence, this increased lethality indicates a severe insufficiency of the local immunity. We studied the capacity of various pathogenic fungi to degrade components of the complement system as a relevant immune evasion mechanism in the CNS.

**Methods:** We studied pathogenic species of Aspergillus, the Pseudallescheria/Scedosporium cluster and some members of the Zygomycetes. The fungi were grown in medium or cerebrospinal fluid (CSF), with or without supplements. Degradation of soluble complement proteins was evaluated by Western Blot. Hyphal opsonization with complement factors was examined by immunofluorescence; cellular expression of surface proteins was quantified by FACS analysis.

**Results:** The growth of Aspergillus spp in CSF resulted in secretion of proteolytic factors which degraded various complement proteins. The extent of proteolysis was dependent on the time period of fungal growth and the Aspergillus species. A. fumigatus, the predominant cause of cerebral aspergillosis, was shown to induce a rather quick and strong degradation. The fungal secretion of proteases correlated with a diminished opsonization of Aspergillus hyphae by complement proteins and the destruction of complement receptor CR3 (CD11b/CD18) on the surface of immune cells. Both opsonization of pathogens and recognition of deposited complement proteins by the corresponding receptors are crucial for an efficient antifungal attack. Additional studies showed that the responsible protease shared many characteristics with the previously described alkaline protease Alp1; furthermore, a mutant lacking Alp1 was unable to degrade complement.

Other Aspergillus species and further pathogenic fungi could also be shown to secrete proteolytic factors. Patient isolates of Aspergillus terreus destroyed complement more rapidly than environmental isolates. In the cluster of Pseudallescheria and Scedosporium, the asexual form Scedosporium generally appeared to secrete more proteolytic activity than the perfect stadium Pseudallescheria. Isolates of Rhizomucor pusillus and Rhizopus microsporus, members of the emerging pathogen group of Zygomycetes, were also found to degrade complement.

**Conclusions:** Aspergillus spp and other pathogenic fungi secrete proteases which can efficiently degrade complement proteins. This may represent a pivotal evasion mechanism especially in the CNS where the complement system is one of only few immune weapons. On the other hand, proteases represent a new therapeutic approach to decrease the lethality of cerebral fungal infections.

## 53. Does Immunosuppressive Therapy Following Kidney Transplantation Induce Accelerated Aging of the Immune System?

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**Background:** Our group has demonstrated that immunosuppressive therapy causes senescence in renal tubular epithelial cells. It is the goal of the present project to investigate whether different types of long term immunosuppressive therapy, namely the calcineurin inhibitors cyclosporine A (CsA) and tacrolimus (FK506), as well as the mTOR-inhibitor rapamycin leads to accelerated irreversible aging of the immunesystem. Frequent and severe courses of infectious diseases and reduced efficacy of vaccination would be the consequence even after dose reduction.

**Methods:** As a first experimental step we investigated proliferation, cell viability, cytokine production (IL-2, IFN-g), telomere length and H2O2 production of human PBMCs exposed to CsA, FK506, or rapamycin in vitro. PBMCs were stimulated by polyclonal activator the phytohemagglutinin (PHA) alone, and in combination with interleukin 2 (IL-2) or interleukin 15 (IL-15), respectively.

**Results:** Treatment with the calcineurin-inhibitors CsA and FK506 leads to decreased proliferation, reduced IL-2 and IFN-g production and shortened telomeres in PBMC. In addition the production of H2O2 is induced by immunosuppressive treatment. Differences between CsA and FK506 can be observed with respect to cell viability as FK506 induces less cell death. Rapamycin does not induce cell death at the tested concentrations. Inhibition of proliferation and cytokine production is less pronounced compared to calcineurin-inhibitors, especially for PBMC stimulated with Interleukin (IL)-15. Induction of H2O2 and shortening of telomeres is also less prominent after treatment with rapamycin compared to calcineurin-inhibitors.

**Conclusion:** Treatment of PBMC with immunosuppressive drugs can be used as an in vitro model for immunosuppressive therapy in order to investigate immunosuppressive effects and toxicity in the context of aging. IL-15 seems to modulate rapamycin-induced immunosuppression. This interaction will be analyzed in detail in further studies.

## 54. Palmar Fibromatosis - a T Cell Driven Disorder?

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Localized hypoxia, cell stress and subsequent activation of immune mechanisms have been shown to be crucial for development of fibrotic diseases. To characterize the role of T cells in palmar fibromatosis, so called Dupuytren`s disease (DD), we processed surgically obtained tissue and blood samples of 100 DD patients (age: 21 to 83 years) for immunohistochemistry, flow cytometry, immunoscope, tissue and cell culture analysis. To identify systemic activation of the immune system of DD patients, serum from 100 affected individuals and 100 healthy volunteers was analysed by ELISA for procollagen III (pIII), circulating immune complexes, soluble ICAM-1 and anti-polymer antibodies (APA). These data were correlated with current lifestyle, health status and illness stage to detect disease promotors.
Statistically significant, 32.9% of DD patients were smokers compared to only 18.6% of the control group ($p=0.047$). From the serological markers, pIII and APA were significantly elevated in DD patients ($p< 0.001$ and $p=0.016$ respectively). Locally, massive infiltration by mononuclear cells ($CD3^+$, CD4>CD8, CD45RO>CD45RA, S100, CD56, CD68, few CD19, mast cells) forming dense perivascular clusters in DD was found. Cytokine profiling of fibromatosis tissue derived T cells showed a Th1 weighted immune response. In addition, we demonstrated a restricted T cell receptor repertoire of intra-lesional T cells - pointing to an antigen-driven process. Thus, T cells seem to play an important role in the onset of palmar fibromatosis.

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## 55. The Role of Endothelial Nitric Oxide Synthase in Ischemia Reperfusion Injury in a Murine Pancreas Transplantation Model

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**Background:** Ischemia-reperfusion-injury (IRI) - related graft pancreatitis is a severe complication following pancreas transplantation, affecting short- and long-term graft survival. Tetrahydrobiopterin (H4B), an essential co-factor of the endothelial nitric oxide synthase (eNOS) and potent antioxidant, was shown to significantly protect from graft pancreatitis in a murine pancreas transplantation model. Since the underlying mechanism is still controversially discussed, the aim of this study was to investigate whether the eNOS enzyme is the main target of H4B using eNOS-/- mice.

**Methods***:* Male syngeneic C57Bl6 (h-2b) mice were used as recipients, and C57Bl6-based eNOS-/- as well as eNOS wild type mice served as donors. Pancreatic grafts were retrieved in a modified no-touch technique, subjected to a 16 h prolonged cold and 45 min warm ischemia time, and transplanted heterotopically into the cervical region via cuff-techinque. Donors were either pre-treated with a single dose 50mg/kg b.w. H4B i.m. or were untreated. Non-transplanted animals of both genotypes - either untreated or treated - served as controls. Microcirculation was analyzed by intravital fluorescence microscopy and quantified by means of functional capillary density. H&E-stained tissue was histologically evaluated by applying the Schmidt pancreatitis score. Intragraft peroxynitrite formation was assessed by nitrotyrosine-immunohistochemistry. Intragraft H4B levels were determined by HPLC. Finally, since IRI of the pancreatic graft in this model was found to be lethal, different groups were tested for recipient survival.

**Results:** Prolonged cold ischemia time resulted in a pronounced breakdown of the microcirculation compared to non-transplanted controls. Independently of their genotype, H4B treated grafts reperfused for 2h displayed markedly improved functional capillary density compared to the corresponding untreated animals ($p=0.09$). In both genotypes, IRI-induced parenchymal edema of the graft, acinar necroses, hemorrhage and fat necroses were decreased following H4B pre-treatment, reaching however only statistical significance in wild type grafts ($p<0.05$). Similarly, nitrotyrosine formation was decreased in both treated groups, reaching statistically significant differences only in wild type organs ($p<0.01$). Compared to non-treated controls, application of H4B significantly enhanced its intragraft levels in all treated groups ($p<0.02$). Finally, a significantly prolonged pancreas recipient survival was observed if H4B was administered, indipendently of the grafts genotype ($p<0.001$).

**Conclusion:** Comparable recipients survival in both genotypes suggests that the eNOS is not the major target of H4B in this IRI-model. However, considering the more prominent decrease of histopathological and immunohistochemical scores in the wild type grafts 2h following reperfusion, eNOS can not be excluded as an additional target for H4B protective effects during early graft reperfusion.

## 56. KIR/HLA Ligand Incompatibility in Kidney Transplantation

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**Background:** The polymorphic family of killer-cell immunoglobulin-likereceptors (KIRs) consists of activating and inhibitory receptors expressed by natural killer (NK) cells and effector T cells which recognize HLA class I ligands. It has been suggested that KIR/HLA Incompatibility exerts beneficial effects in hematopoietic stem cell transplantation.

**Methods**: To elucidate whether certain receptor-ligand combinations between recipient KIR and donor HLA antigens lead to enhanced alloreactivity of NK cells associated with acute rejection (aRx) post kidney transplantation we analyzed the entirety of matches/mismatches between KIR genes and known HLA ligands for aRx patients (n=105) compared to patients with stable renal function (n=119).

**Results:** Whereas HLA-C ligand incompatibility between donor and recipient has no influence on aRx, grafts derived from donors homozygous for HLA-C group 2 alleles seem to demonstrate a better outcome (p=0.052). Additionally a higher number of inhibitory receptors in the recipient´s genotype (p=0.042), a significant higher number of matches for the receptors KIR2DL2/DS2 (p=0.004) as well as a higher number of mismatches for KIR2DL3 (p=0.014) could be observed for patients with stable renal function.

**Conclusion:** Our data illustrate that certain KIR/HLA class I ligand combinations between donor and recipient might influence graft short-term outcome following renal transplantation.

## 57. Role of *Hfe* in the regulation of macrophage iron homeostasis and immune response following *Salmonella* Typhimurium infection

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Mutations in *HFE* cause classical hemochromatosis, a hereditary metabolic disorder characterized by parenchymal iron deposition and macrophage iron depletion. The role of HFE in the regulation of iron homeostasis under inflammatory conditions and in the control of infectious agents however, is incompletely understood.

Using $Hfe^{+/+}$ and congenic $Hfe^{-/-}$ mice in a model of *Salmonella enterica* serovar Typhimurium infection, we found animals of either genotype to appropriately induce IL-6 and hepcidin antimicrobial peptide (Hamp) following systemic infection resulting in adequate hyposideremia and hyperferritinemia characteristic of the acute-phase response. Of note, $Hfe^{-/-}$ mice displayed reduced spleen and macrophage iron content paralleled by increased production of the siderophore-binding antimicrobial peptide lipocalin 2 (Lcn2). The increased levels of Lcn2 detected in $Hfe^{-/-}$ mice and macrophages, respectively, were attributable to increased HIF-1α and NF-κB p65 activation and resulted in improved control of WT but not siderophore-deficient *Salmonella* replication. This effect which was abrogated upon inhibition of HIF-1α and p65, upon ablation/neutralization of Lcn2 or supplementation of iron to infected macrophages. Moreover, synthetic Hamp impaired while recombinant Lcn2 promoted bacterial elimination within macrophages.

Our data suggest that $Hfe^{-/-}$ mice show appropriate adaptation of iron homeostasis to invasive *Salmonella* infection and produce higher amounts of the iron-capturing peptide Lcn2 via a HIF and NF-κB-dependent mechanism. This may results from reduced iron levels within $Hfe^{-/-}$ macrophages and appears to harbor an immunological advantage towards infections with *Salmonella*.

## 58. Nramp1 Induces Lipocalin-2 Production Leading to Enhanced Killing of S. Typhimurium

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In mice, the expression of the phagolysosomal protein Nramp1 (natural resistance associated macrophage protein 1, Slc11a1) confers host resistance to several intracellular pathogens, such as Salmonellae, Mycobacteria and Leishmania. Nramp1 is expressed in phagocytic cells and acts as a transporter for protons, iron and other divalent cations. The expression of Nramp1 is associated with enhanced activity of pro-inflammatory pathways (such as the formation of nitric oxide) as well as down-regulation of the anti-inflammatory cytokine interleukin-10. Lipocalin-2 (Lcn2) is a small antimicrobial peptide, which exerts bacteriostatic effects by depriving bacteria of the essential nutrient iron. Lcn-2 binds and contains iron loaden bacterial siderophores, the most important iron source for these microorganisms.
Using RAW264.7 murine macrophages stably transfected with functional (RAW-37) or non-functional (RAW-21) Nramp1, we investigated the influence of Nramp1 expression on Lcn2 production. We found that Nramp1 function leads to up-regulation of both, mRNA and protein levels, of Lcn2 upon stimulation of macrophages with IFN-gamma and LPS. Upon infection of macrophages with S. typhimurium addition of a neutralising anti-Lcn2-antibody abolished the ability of Nramp1-expressing cells to control bacterial growth. Furthermore, Lcn2 (Nramp1 expression) did not affect the growth of intramacrophage Salmonellae carrying a mutation of the siderophore enterobactin. Inhibitor experiments and electro mobility shift assays showed that enhanced NFkappaB binding activity leads to upregulation of Lcn2 in Nramp1-functional RAW-37 cells.
Taken together, Nramp1 exerts a novel anti-microbial function via induction of lipocalin-2 production and deprivation of the essential nutrient iron for intracellular pathogens.

## 59. IL-32: A New Pro-inflammatory Cytokine Involved in HCV-related Liver Inflammation and Fibrosis

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**Background:** Interleukin 32 (IL-32) is a recently described proinflammatory cytokine that activates the p38 MAPK and NF-κB pathways, thereby inducing other proinflammatory cytokines such as IL-1β, IL-6, and TNF-α. The present study investigated the role of IL-32 in patients with chronic hepatitis C virus (HCV) infection. To assess whether IL-32 might impact hepatocyte immunobiology, in vitro studies were performed to evaluate regulation of endogenous and responsiveness to recombinant IL-32.

**Methods:** The study included 90 consecutive patients with untreated chronic HCV infection. Hepatic IL-32 expression was quantitated by real-time PCR and immunohistochemistry to assess the relationship between IL-32 and hepatic steatosis, fibrosis, and inflammation. In vitro we studied the effects of IL-1b, TNF-α with or without IFN-α on IL-32 expression in the human hepatoma cell lines Hep3B and Huh7, as well as on primary CD14-positive monocytes. IL-32 was determined by quantitative real-time PCR and confirmed by western blot. Finally, we determined the effect of IL-32 on HCV replication using a HCV replicon system in both IL-32-overexpressing and IL-32-silenced Huh7 cells.

**Results:** Highly significant positive correlations between hepatic IL-32 mRNA expression and liver fibrosis, smooth muscle actin (SMA) area, hepatic steatosis, liver inflammation (Ishak score), and alanine aminotransferase (ALAT) were observed. IL-32 protein expression as determined by immunohistochemistry was positively correlated with portal inflammation, SMA area, and ALAT. In vitro, IL-1ß and TNF-α significantly induced IL-32 expression in Hep3B and Huh7 cells. IFN-α exerted a significant additive effect on TNF-induced but not on IL-1-induced IL-32 expression. This effect was even more impressive in CD14-positive monocytes. Overexpression or silencing of IL-32 did not affect replication of HCV-reporter chimeras in transfected Huh7 Lunet cells as determined by luciferase assays performed at 24, 48, 72, and 96 hours posttransfection.

**Conclusions:** Our results suggest that IL-32 might be a novel pro-inflammatory cytokine involved in hepatic inflammation and liver fibrosis in chronic HCV infection. IL-32 is produced by hepatocytes and up-regulated by proinflammatory cytokines.

## 60. IgG Antibody Avidity after Varicella-Zoster-Virus (VZV) Vaccination in Solid Organ Transplant Recipients (SOT)

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**Background:** Primary infection with Varicella-Zoster-Virus (VZV) usually causes a mild disease in healthy individuals but severe complications (such as secondary bacterial infections, pneumonia, encephalitis and death) can occur in immunocompromised patients. Prophylaxis can be achieved with a live-attenuated vaccine. This is of particular importance for seronegative transplant candidates. Immunity against VZV is a function of both, humoral and cellular immunity. So far, immunity is usually determined by evaluation of VZV-specific IgG antibodies.

**Methods:** Serum IgG antibody concentrations were determined by ELISA according to the manufacturers' instructions. Serum IgG antibody avidity was determined by using an adapted ELISA with urea treatment to remove low avidity antibodies. Relative avidity index (RAI) was defined by the extinction with urea treatment divided by the extiction without urea treatment multiplied by one hundred.

**Results:** There was no difference for IgG antibody concentrations between SOT 956±818 (820) and HC 929±711 (800). SOT patients showed significantly lower IgG antibody avidity 72±20 (79) compared to vaccinated and wild-type infected ($p<0.001$) healthy controls 90±7 (90). Particularly, lung transplant recipients 73±20 (74) showed significantly lower RAI compared to HC ($p<0.001$).

[IgG concentrations are given in mIU/ml mean±standard deviation (median)]

[IgG antibody avidity is given in % mean±standard deviation (median)]

**Conclusion:** Despite normal VZV-specific IgG concentrations, the IgG avidity was significantly reduced in SOT patients. Although the clinical importance of these findings is still unclear, it may be suggested that IgG antibody avidity in SOT recipients may serve as an additional marker to evaluate humoral immunity against VZV. It appears from clinical cases, that not only the humoral but also the cellular immunity against VZV should be consistently monitored to assess waning immunity under immunosuppressive treatment. This approach is desirable to estimate the risk of severe varicella disease after exposure or endogenous VZV reactivation in these patients.

## 61. Humanized Mouse Models for HIV Gene Therapy

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**Background:** Previously, the infusion of T cells transduced with a retroviral vector (M87o) that expresses a membrane-anchored HIV entry inhibitor (maC46) was tested in a Phase I clinical trial. Gene-modified autologous T cells were infused into 10 HIV-infected patients with advanced disease and multidrug resistant virus during antiretroviral combination therapy. Transfer of gene-modified cells was safe, led to sustained levels of gene marking and induced some improvement of immune functions. However, the low level of gene marking and the lack of a substantial in vivo accumulation of gene-protected cells prompted us to return to the bench and further improve our strategy. As murine cells are not suitable to HIV infection, here, the efficacy of maC46 is further investigated in humanized mouse models.

**Methods:** In a first model (human immune system = HIS mouse model) newborn immune deficient Rag-2/gamma chain double knockout mice were repopulated with gene-modified human $CD34^+$ cord blood hematopoietic stem cells. Two further models only use human mature T cells as graft. These cells are either transplanted into immune deficient NOD/SCID/gamma chain knockout mice (T cell mouse model) or into wild type mice after lethal irradiation and radioprotection with bone marrow cells from an immune deficient mouse (Trimera model). As the Trimera model is based on a wild type mouse, secondary lymphoid structures (eg. lymph nodes) can be found in the mice. The transplanted human T cells are able to migrate into these structures and thereby T cell homeostasis in these animals should be improved.

**Results:** In the HIS mouse model, mice were successfully repopulated with human immune cells. It is especially interesting that human $CD4^+$ T cells, the target cells of HIV, engrafted in the animals. Repopulated mice were infected with HIV by intraperitoneal injection. Infection with HIV led to a drop of human $CD4^+$ T cell counts and a peak of viral load in plasma. But most likely due to a lack of homeostatic proliferation of human T cells in this model, no clear selection of maC46 expressing $CD4^+$ T cells could be found. Transplantation of mice with gammaretroviral or lentiviral transduced human T cells in the T cell mouse model also led to repopulation with gene modified human $CD4^+$ T cells in the blood. The cells were infected with HIV either in vitro prior transplantation or in vivo after repopulation. In all experiments there was a substantial increase of $maC46^+$ expressing cells within the human $CD4^+$ T cells in blood as well as in spleen apparently due to the selective pressure of ongoing HIV infection. This increase of $CD4^+$ T cells was neither seen in uninfected control mice nor with a control vector.

**Conclusion:** Further studies in these models with the maC46 peptide as well as with iSAVE will allow us to analyze the conditions that determine efficacy of immuno/gene therapy for HIV-infection.

## 62. SidL, an Acetyltransferase Involved in Biosynthesis of the Intracellular Siderophore Ferricrocin in *Aspergillus Fumigatus*

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**Background:** Virtually all organisms require iron as indispensable cofactor for various metabolic processes. The opportunistic fungal pathogen *Aspergillus fumigatus* produces two major siderophores (low molecular-mass ferric iron chelators): it excretes triacetylfusarinine C for iron uptake and accumulates ferricrocin for intracellular iron storage. Biosynthesis of both triacetylfusarinine C and ferricrocin has previously been shown to be crucial for virulence of *A. fumigatus.*

**Methods:** In order to characterize the function of a putative transacetylase Afu1g04450, the gene was replaced and the resulting deletion strain was analyzed with regard to phenotype, siderophore production and growth under iron depleted and repleted conditions. Further on we tagged the transacetylase with gfp to lozalize the protein.

**Results:** Here, we report the functional characterization of a new component of the fungal siderophore biosynthetic machinery Afu1g04450, termed SidL. SidL is conserved in siderophore-producing but not non-siderophore producing ascomycetes. The C-terminal half of SidL shows similarity to acetylases involved in bacterial siderophore biosynthesis, e.g. *Escherichia coli* IucB (a hydroxylysine acetylase required for aerobactin biosynthesis) and PvdY (a hydroxyornithine acetylase required for pyoverdin biosynthesis), and the hydroxyornithine:anhydromevalonyl coenzyme A-transacylase SidF that is essential for triacetylfusarine C biosynthesis. Deletion of *sidL* in *A. fumigatus* reduced ferricrocin biosynthesis during iron starvation and blocked ferricirocin biosynthesis during iron-replete growth. Furthermore, *sidL*-deficiency blocked conidial ferricrocin accumulation under strict iron-replete conditions but not when mycelia were transferred from iron-depleted to iron-replete conditions before sporulation. In contrast, SidL-deficiency had no effect on triacetylfusarinine C production. The expression of *sidL* was affected neither by iron availability nor the iron regulator SreA.

**Conclusion:** Taken together, these data show that SidL is a constitutively expressed hydroxyornithine acetylase involved in ferricrocin biosynthesis. Moreover, the data indicate the existence of a second hydroxyornithine acetylase, the activity of which is induced by iron starvation. This study identified a novel component of the fungal siderophore biosynthetic machinery and revealed unexpected complexity.

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## 63. Intracellular Signaling Pathways as Targets for the Prevention of Ischemia/Reperfusion-Induced Damage during Solid Organ Transplantation

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**Background:** Excessive production of reactive oxygen species (ROS) is an integral part of the cellular stress response and a major contributing factor to the development of ischemia-reperfusion injury (IRI). In particular mitochondrially-produced ROS are critical for the initiation and progression of IRI, which restricts the pool of donor organs and results in elaborate follow up treatments. The use of antioxidants showed very limited clinical benefit and novel treatment concepts are needed. In several *in vivo* and *in vitro* (hypoxia/reoxygenation, HR) models we observed a marked increase in the activity of the stress kinase p38 and inhibiting p38 in HL-1 cardiomyocytes showed protective effects during HR. Here we further defined the contribution of p38 to IR- and HR-induced damage and established the conditions for the *in vivo* testing of p38 inhibitors.

**Methods:** Kidney clamping and kidney transplantation in Wistar and Lewis rats were used for the study of IR. Hypoxia/reoxygenation were analyzed in various cell models (HL-1 cardiomyocytes, HUVEC, glomerular mesangial cells). Intracellular signaling was monitored in cell or tissue lysates using phosphorylation-specific antibodies. Mitochondrial ROS and $Ca^{2+}$ levels were determined by imaging of cells pre-labeled with MitoTracker Red CM-H2XRos and Rhod-2, respectively. ROS/NOS-induced damage in tissue lysates was visualized by 3-nitrotyrosine specific antibodies.

**Results:** Use of the p38 inhibitors SB203580 and BIRB-796 in HL-1 cells prevented HR- induced ROS production, $Ca^{2+}$ overload and cell death. The expression pattern of all p38 isoforms was established in HL-1 cells and siRNA-mediated knockdown of the predominant isoform p38$\alpha$ reduced ROS production, confirming the critical role of p38 in modulating mitochondrial ROS levels. Preliminary evidence suggests the involvement of MAPKAP kinase 2 (MK2) rather than the transcription factor ATF-2 downstream of p38 in ROS induction. As observed previously in a heterotopic heart transplant model reperfusion following kidney clamping or transplantation was marked by a profound increase in the activity of p38, its upstream kinases MKK3/6 and the effector MK2. Application of the p38 inhibitor BIRB-796 in the kidney clamping model showed dose-dependent inhibition of p38 without affecting the related kinases JNK and ERK. Inhibition of p38 *in vivo* also efficiently blocked ROS/NOS formation.

**Conclusion:** Inhibition of p38 during IR and HR prevents several processes, which are essential for the development of IRI.

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## 64. GliT Protects Aspergillus Fumigatus against the Harmful Effects of Gliotoxin

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**Background:** Gliotoxin, and other related molecules, are encoded by multi-gene clusters and biosynthesized by fungi using non-ribosomal biosynthetic mechanisms. Almost universally described in terms of its toxicity towards mammalian cells, gliotoxin has come to be considered as a component of the virulence arsenal of the fungal pathogen Aspergillus fumigatus.

**Methods:** Here we report the functional characterisation via gene-deletion of a putative thioredoxin reductase encoded by gliT within the gliotoxin biosynthetic cluster.

**Results:** Expression of gliT is subject to regulation by the transcriptional activator GliZ and gliotoxin. Deletion of gliT is detrimental for growth only in the presence of exogenously added gliotoxin, which can be cured by supplementation with reduced glutathione. GliT is not essential for virulence of A. fumigatus in larvae of the greater wax-moth Galleria mellonella and is localised in the cytoplasm and in the nucleus. The potential autoprotective role of GliT was investigated further by heterologous expression of gliT in Aspergillus nidulans.

**Conclusion:** GliT confers resistance to gliotoxin, making it a valuable tool for transformation of fungi lacking an ortholog of gliT.

## 65. Investigation of Ischemia/Reperfusion Injury on Composite Tissue Allografts

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**Background:** The effect of cold ischemia (CI) as well as preservation solutions on composite tissue allografts remain undefined. We herein investigate the effect of cold tissue storage and preservation with HTK and UW in a rat hind limb transplant model.

**Methods:** LEW rat limbs were flushed and stored for 0, 2, 10, 30 and 40h in HTK or UW preservation solution. Skin, muscle, bone and nerve biopsies were taken at any time point for H&E histology. After transplantation (subsequent to 2h, 10h or 30h of CI), limbs were analyzed for morphological alterations by histomorphology and confocal microscopy at 1 and 10 days. Histology of all tissues was rated using a previously established scoring system: 0 no alterations; 1 mild alterations; 2 severe alterations; 3 necrosis.

**Results:** Appearance and histology of skin, bone and nerve remained unaltered at any time point during preservation. Histomorphologic changes of muscle fibers were observed in some biopsies regardless of preservation solutions and time of CI. Clinically, limbs were most affected in the 30h CI-group at 10 days after transplantation. 2h, 10h and 30h of CI and subsequent reperfusion did not cause alterations in histomorphology of skin and muscle at 1 day. At 10 days, skin showed a mild lymphocytic infiltrate in all samples. In muscle a mild lymphocytic infiltrate was found in groups of 10h of CI, after 30 h of CI highly affected and necrotic muscle fibers were obtained. Nerve showed alterations, vasculopathy and an infiltrate and was hyper cellular, especially at 10 and 30 h of CI. Bone was not affected at all, however, secondary infection was observed in some samples, regardless of preservation solution and CI time. Overall, tissues were more affected of CI (after 2h, 10h and 30h) when flushed and stored in UW than HTK (skin: 0,4 vs 0,0 / 1,2 vs 0,3 / 2,0 vs 1,0 | muscle: 1,4 vs 0,5 / 2,0 vs 1,3 / 3,0 vs 2,5 | Nerve: 1,2 vs 1,7 / 1,8 vs 1,8 / 2,0 vs 1,0). Results of confocal microscopy revealed increased cell damage the longer the time of CI, but did not allow comparison of the two preservation solutions.

**Conclusions:** Cold ischemia causes mild histomorphologic alterations on muscle, but not on skin, nerve and bone. Most severe histomorphological changes due to ischemia reperfusion injury can be observed in nerve and muscle at 10 days in transplanted legs with an advantage of HTK over UW for tissue preservation. Further studies are needed to specify histomorphologic alterations.

## 66. Increased Plasma Phenylalanine to Tyrosine Ratio in HIV-1 Infection and Correction Following Effective Antiretroviral Therapy

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Neuropsychiatric symptoms like cognitive impairment or depressive mood represent frequent symptoms in patients with progressed HIV-1 infection. Impairment of serotonergic and/or dopaminergic neurotransmission may play a role in their precipitation. Accelerated degradation of the essential amino acid tryptophan due to increased expression of indoleamine 2,3-dioxygenase is common in patients with HIV-1 infection and may influence serotonin availability. Accordingly many patients respond rather well to treatment with selective serotonin reuptake inhibitors (SSRI), but others do not, which suggests that another background like an altered metabolism of the catecholamines dopamine, adrenaline (epinephrine) and noradrenaline (norepinephrine) could be important as well. Several years ago, higher serum/plasma concentrations of the essential amino acid phenylalanine (phe) have been documented in patients with HIV-1 infection. They may relate to a diminished conversion of phe to tyrosine (tyr) by the enzyme phenylalanine-hydroxylase (PAH). PAH is rate-limiting in the biosynthesis of dopamine, and impaired PAH activity is reflected by an increased phe to tyr ratio (phe/tyr). In this study, plasma phe/tyr was measured in 107 patients with HIV-1 infection before and after 12 months of effective antiretrovial therapy (ART). Results were compared with $CD4^+$ cell counts, HIV-1 RNA levels and concentrations of immune activation marker neopterin.

Before ART, phe/tyr was mean ± S.D.: 0.99 ± 0.57 µmol/µmol and correlated significantly with plasma and urine neopterin concentrations (both $p < 0.001$) and less strongly with HIV-RNA levels and $CD4^+$ counts (both $p < 0.05$). After ART, phe/tyr dropped which was due to a decline of phe concentrations from and a concomitant increase of tyr concentrations (all $p < 0.001$). In parallel, significant reductions of plasma and urine neopterin concentrations were observed during ART.

Increased phe/tyr is frequent in patients with HIV-1 infection and is related to immune activation. ART was found to decrease phe/tyr and this change could indicate and influence on PAH activity.

Increase of phenylalanine is probably due to diminished activity of PAH which may relate to a role of ROS in diminishing necessary enzyme cofactor 5,6,7,8-tetrahydrobiopterin. Any relevance for the neuropsychiatric abnormalities in patients still needs to be shown. Future studies might be able to show whether the decline of phe/tyr under ART may concur with the often improved neuropsychiatric status in treated patients.

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## 67. Influence of Immunosuppressive Agents on Tryptophan Degradation and Neopterin Production in Human Peripheral Blood Mononuclear Cells

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**Background:** The antiproliferative and tolerance-inducing enzyme indoleamine-2,3-dioxygenase (IDO) degrades the essential amino acid tryptophan to kynurenine. IDO is stimulated within cellular immune response preferentially by Th1-type cytokine interferon-gamma (IFN-gamma). IDO activity can be estimated by calculating the ratio of the first product kynurenine to substrate tryptophan (kyn/trp). GTP-cyclohydrolase I is induced in parallel to IDO and gives raise to the production of neopterin in human monocyte-derived macrophages and dendritic cells. This in vitro study investigated the effects of immunosuppressants tacrolimus (FK506, Prograf), cyclosporine A (CsA, Sandimmune), prednisolone, sirolimus (Rapamune), mycophenolate-mofetil (MMF, CellCept), prednisolone and methylprednisolone (Urbason) on freshly isolated peripheral blood mononuclear cells (PBMC) from healthy blood donors.

**Methods:** PBMC were incubated with accelerating doses of immunosuppressants and were either left unstimulated or after 30 minutes were stimulated with T-cell mitogen phytohaemagglutinin (PHA). Concentrations of tryptophan and kynurenine were measured in supernatants after 48 hours of stimulation. Neopterin concentrations were measured for comparison.

**Results:** IDO-activity and neopterin formation were significantly higher in PHA-stimulated PBMC than in unstimulated cells. FK506, sirolimus, CsA and methylprednisolone dose-dependently inhibited tryptophan degradation and neopterin production. Whereas FK506, CsA and sirolimus showed significant inhibition at concentrations as low as 0.1 mcg/ml, prednisolone and methylprednisolone required concentrations higher than 10 mcg/ml to suppress tryptophan degradation. MMF seemed to suppress neopterin formation more efficiently than IDO-activity. In unstimulated cells, MMF, prednisolone, FK506, sirolimus, CsA and methylprednisolone at higher doses also inhibited tryptophan degradation and neopterin production.

**Conclusion:** Overall the investigated immunosuppressants are effective to inhibit IDO activity and neopterin production in a similar way and in a dose-dependent manner. However, there were some distinct effects when comparing the different compounds.

## 68. Complement Allotypes Associated with Typical Hemolytic Uremic Syndrome

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**Background:** Typical hemolytic uremic syndrome (HUS), a severe renal disease, is mainly caused by infections with enterohemorrhagic *E.coli* (EHEC) strains. About 10% of all HUS cases are termed atypical HUS (aHUS), observed as familial or sporadic forms, which are not dependent on bacterial infections and where recurrences are possible. Complement plays an important role in aHUS, a mutation in a regulator is mainly the cause of the disease. Recently, an involvement of complement has also been described for typical HUS.

The aim of our study was to analyse the influence of a protein polymorphism of complement C7, which has been identified based on the reactivity of an allospecific mouse monoclonal antibody, and Factor H (FH), a central regulator of the alternative pathway which acts as cofactor for factor I, on the risk of EHEC infections and development of typical HUS.

**Methods:** Eighty-three serum samples of typical HUS patients were tested for allotypes of the C7 M/N and the FH Y402H polymorphisms. Typing of C7 M/N was performed as published (Würzner et al. 1990). For determination of the allotypes of the FH Y402H polymorphism an ELISA based test kit was used (Hycult, Uden, The Netherlands). A Fisher's exact test and a chi- square- test were performed to compare the data of healthy controls with typical HUS patients for C7 M/N and FH Y402H polymorphisms, respectively.

**Results:** C7 M/N and FH Y402H allotyping of the 83 sera from HUS patients investigated in this study showed that there was no significant difference ($p > 0.01$) in the distribution of allotypes of HUS patients when compared to those of healthy individuals.

**Conclusion:** In contrast to a higher frequency of FH 402H in age- related macular degeneration, a disease also affecting the kidney, in this study of C7 M/N and FH Y402H polymorphisms in HUS patients, there is no association to susceptibility to typical HUS. Further studies will show whether other polymorphic variants of C7 and FH are associated with diarrhea positive hemolytic uremic syndrome.

## 69. TDS-GCMS Analysis of Volatile Metabolites for the Detection and Monitoring of Diseases

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**Background:** The objective of our work was to identify volatile organic compounds (VOCs) in the human breath, which can serve as sensitive and specific markers for the early disease detection detection. For the development and validation of our experimental approach we used lung cancer as a model disease. The cellular origin of VOCs found in breath of cancer patients is unclear and they may derived from at least three sources, cancer cells, cells of the immune system and infectious agents. Here we set out to confirm the presence of truly tumor cell-derived VOCs.
**Methods:** Four lung cancer cell lines (A549, NCI-H2087, NCI-H1666 and CALU-1), healthy primary lung epithelial cells and fibroblasts from the human dermis (hFB) have been compared by gas chromatography mass spectrometry (GC-MS). The lung cancer cells used either express oncogenic, mutated B-RAF (NCI-H1666, NCI-H2087) or K-RAS (A549, CALU-1). 100x106 cells were incubated in a sealed fermenter for 18-21 hours and prior to GC-MS analyses samples from the headspace were collected and preconcentrated by adsorption on solid sorbents. Then sampled VOCs were thermodesorbed and analyzed by GC-MS.
**Results:** The investigated cell lines and primary cells all showed release of VOCs and consumption of VOCs, with the exception of NCI-H1666 cells, which lacked a clear release of VOCs. We identified VOCs that behaved similarly in normal and transformed cells. For instance, concentrations of 2-pentanone and 2,4-dimethyl-1-heptene were found to be increased in normal and A549 cells. The same was found for 2,3,3-trimetylpentane in control and CALU-1 cells. In addition, methyl tert-butyl ether and ethyl tert-butyl ether were elevated in A549 cells and one of the untransformed cell lines. However, especially branched hydrocarbons and alcohols were released at higher rates from untransformed cells. A big variety of predominantly aldehydes and the ester n-butyl acetate were found at decreased concentrations in the headspace of all cell lines tested compared with medium controls. Again, more different aldehydes were found to be decreased in hFB and HBEpC cells compared with A549 cells and 2-butenal was metabolized exclusively by both control cell lines.
**Conclusion:** These data suggest that certain groups of VOCs may be preferentially associated with the transformed phenotype of cancer cells. Moreover, in the course of our work we generated the technical platform, which is now adapted for the analysis of pathogen-derived VOCs to obtain a comprehensive picture of potential breath-derived VOCs.

## 70. Reduction of the Expression of Complement Regulators CD46 and CD55 on Tubulus Epithelial Cells by Shiga Toxin 2

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**Background:** Shiga toxin 2 (Stx2) is one of the most important virulence factors of enterohaemorrhagic Escherichia coli (EHEC). Infections with this bacterium are reckoned to be the main reason for haemolytic-uremic-syndrom (HUS). In addition to the EHEC-induced HUS there are inherited forms of HUS caused by mutations in regulators of the complement system, such as the soluble factor H or the cell-surface associated CD46 (MCP). In addition, CD55 (DAF) and CD59 are also cell-surface regulators. Both CD46 and CD55 play a role in the deactivation of the C3- and C5 convertases, whereas CD59 modulates membrane attack assembly. The aim of the study was to investigate whether or not treatment with Stx2 changes the expression of CD46, CD55 or CD59 on tubulus epithelial cells.

**Methods:** The Stx2 was purified from the culture supernatant according to standard procedures. A human kidney cell line (HK-2 cells) in middle passage number was incubated with 0.2µg/ml Stx2 at 37°C and 5%CO2 in a 75cm² filtertop cell culture flask for 24h. As control group the Stx2 was replaced by sterile PBS. FACS was done according to standard protocols using a BD FACS CantoII. The results were calculated from the measurements of seven independent FACS experiments.

**Results:** The HK-2 cells show a significant reduction in CD46 and CD55 expression (both $<0.05$) after treatment with Stx2. CD59 shows no significant downregulation with identical treatment.

**Conclusion:** Together with our previous findings, showing that Stx2 activates the complement system, we draw the conclusion that Stx2 additionally reduces the complement regulators CD46 and CD55 on the cell surface so that the cells become more vulnerable against complement attack.

## 71. P14 - a Potential Novel Host Defense Factor

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**Background:** Signal specificity in the MAPK cascade is regulated by the assembly of different scaffold complexes at distinct subcellular locations. The scaffold protein MP1 (MEK1 partner) is localized to late endosomes by the adaptor protein p14. Conditional gene disruption of p14 in mice demonstrate that the late endosomal p14/MP1-MEK1 signaling complex is required to control endosomal traffic and cellular proliferation during tissue homeostasis (Teis D. et al, JCB, 2006). Interestingly, a novel human primary immunodeficiency syndrome was identified which is due to a homozygous single point mutation in the human p14 gene, leading to reduced p14 protein levels. Phenotypically, these patients suffer from severe neutropenia and defective lysosomal function in granulocytes and monocytes (Bohn G. et al, Nat. Med., 2007). Therefore, we were specifically interested in addressing the molecular function of the late endosomal scaffold complex p14/MP1 in innate immunity in vivo and in vitro.

**Methods:** For this purpose, we generated conditional knock out mice expressing the Cre recombinase under the control of the lysozyme M promoter which allowed us to specifically delete p14 in the monocyte/macrophage cell lineage (LMCp14-/- mice). As an Infection model we have used bone marrow derived macrophages which were exposed to Salmonella thyphimurium.

**Results:** In an infection model LMCp14-/- mice were more susceptible to the pathogen Salmonella tm compared to their wild type littermates as could be judged by the higher bacterial load in spleen and liver. Furthermore, LMCp14-/- mice displayed fewer and less defined granulomas in these organs. Interestingly, an increased number of intracellular bacteria was observed in macrophages from LMCp14-/- animals in vivo and in bone marrow derived macrophages in vitro. P14 does not play a role in caspase 1 induced apoptosis or in the phagocytic uptake of bacteria. However, p14 is needed for the late endosomal ERK activation which enables the targeting of Salmonella tm into the phagolysosomal pathway. Furthermore, p14 is involved in the recruitment of the NADPH oxidase subunit p47phox to late endosomes. Indeed, the loss of p14 influences the endo/phagosomal system, thereby providing a better niche for the survival and replication of bacteria. Additionally, the loss of p14 results in reduced expression of pro-inflammatory signals as well as antimicrobial factors.

**Conclusion:** The late endosomal scaffold complex p14/MP1 governs the trafficking of Salmonella tm into the endo/phagolysosomal system, thus ensuring the efficient clearance of intracellular bacteria in macrophages.

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## 72. Clinical Target-Site Pharmacokinetics of Antifungal Drugs

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**Background:** Invasive fungal infections remain a major cause of morbidity and mortality in high-risk patients. Amphotericin (AMB) lipid formulations are an important cornerstone of antifungal therapy with reduced toxicity: liposomal AMB (LAMB, Ambisome®), AMB colloidal dispersion (ABCD, Amphocil®) and AMB lipid complex (ALBC, Abelcet®). Voriconazole (VRC, Vfend®) is a second-generation triazole with a broad antimycotic spectrum, which is the drug of choice for treatment of invasive aspergillosis. Antimycotic drug concentrations at target-site can be essential for clinical response. Concentrations of antifungal drugs were determined in human tissue and body fluids.

**Methods:** AMB levels in human tissue of 20 patients (LAMB: n=7, ABCD: n=13), epithelial lining fluid of 44 patients (LAMB: n=11, ABCD: n=28, ABLC: n=5), pleural effusion of seven patients (LAMB: n=1, ABCD: n=5, ABLC: n=1), ascites of three patients (LAMB/ABLC: n=1, ABCD: n=2) and bile fluid (ABCD: n=1) were analysed after administration of an AMB lipid formulation. VRC levels in human autopsy samples were determined in 8 patients. Samples were purified by solid phase extraction and antifungal substances were quantified by high-pressure liquid chromatography technique.

**Results:** The three AMB lipid formulations show remarkable differences in their distribution behaviour into different compartments. Total AMB levels in pleural effusion ranged from 0.02µg/mL to 0.43µg/mL. Mean levels of 0.22±0.09 and 0.05±0.05µg/mL were determined in ascites for the liberated and the lipid-formulated AMB fraction, respectively. Liberated AMB could be detected in all samples of pleural effusion (0.02-0.40µg/mL), ascites (0.15-0.36µg/mL) and bile fluid (0.78µg/mL). Concentrations of the lipid-associated fractions were very low in body fluids (<0.11µg/mL). The highest concentrations in ELF were found after treatment with LAMB (mean 1.60µg/mL) and ABLC (mean 1.29µg/mL) in contrast to ABCD (mean 0.38µg/mL). Total AMB levels in body fluids were significantly lower than the total concentrations in plasma (0.40-6.91µg/mL) and total tissue concentrations. The highest AMB levels in human tissue were found in liver and spleen, followed by kidney, lung, myocardium and brain. After treatment with ABCD and ABLC, concentrations in lung tissue were higher than after LAMB (mean LAMB: 11.63µg/g, ABCD: 32.62µg/g, ABLC: 31.96µg/g).

The tissue levels (mean±SEM) of VRC amounted to 7.53±1.10µg/g in the lung, 9.45±1.73µg/g in the brain, 14.96±3.68µg/g in the liver, 10.62±2.41µg/g in the myocardium, 7.49±1.16µg/g in the kidneys and 7.24±1.59µg/g in the spleen.

**Conclusion:** AMB lipid formulations displayed markedly different disposition patterns at target-site. VRC is detectable in most tissues, including the lung, already after the first administration. Highest VRC concentrations are reached in the liver.

## 73. HapX is Involved in Maintainance of Iron Homeostasis and Virulence of Aspergillus fumigatus

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**Background:** Consistent with iron playing a crucial role in virulence, we have previously shown that biosynthesis of siderophores (iron chelators involved in uptake, storage and intracellular distribution of iron) is essential for virulence of A. fumigatus. The characterization of the fungal iron metabolism might aid improvement of diagnosis and treatment of fungal infections.
**Methods:** Generation of fungal gene deletion strains followed by phenotyping.
**Results:** During iron replete conditions, siderophore biosynthesis is repressed by the GATA factor SreA. Here we report the characterization of a second iron regulator, the bZIP transcription factor HapX. SreA and HapX are interconnected by a regulatory feedback loop: SreA repressed expression of hapX during iron sufficiency and, vice versa, HapX repressed expression of sreA during iron starvation. During iron starvation, inactivation of HapX resulted in derepression of iron-dependent pathways (e.g. the mutant strain displayed accumulation of the iron-free heme precursor protoporphyine IX) but reduced production of extra- and intracellular siderophores. Moreover, the hapX deletion mutant displayed significantly reduced virulence in a murine model of aspergillosis.
**Conclusions:** This study demonstrates the crucial role of HapX in iron regulation and virulence of A. fumigatus. Deleterious consequences of inactivation of SreA and HapX are strictly confined to iron replete and -depleted conditions, respectively. Consequently, attenuation of virulence by inactivation of HapX, but not of SreA, underlines that A. fumigatus faces iron-limited conditions during mammalian infection.

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## 74. Improved Somatic Cell Genetic Loss-of-function Tools: AAV-vector-based Conditional Knock-out and Lentiviral Conditional RNAi

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(1) Genetic engineering of human somatic cells is an essential tool for gene therapy and for the creation of genetically defined cell lines for basic research and drug screening. Gene-targeting to obtain a loss- or gain-of-function allele in human somatic cells is, however, less efficient and more time-consuming than in murine embryonic stem cells. For gene-targeting in human somatic cell lines, we have established a method, which is based on the viral delivery and gene-specific insertion of an excisable gene-trap cassette. After excision, tetracycline repressor binding sites are left behind in an intron of the targeted gene to render it suppressible by tetracycline-inducible transcriptional repressors. To demonstrate the versatility of this approach, we have generated an adeno-associated viral (AAV) vector, containing a gene-trap cassette flanked by 1kb homology flanks for targeted insertion into intron 1 of the human HPRT gene. After infection, HCT116 cells were selected for the presence of the targeting vector and loss of HPRT function using G418 and 6-thioguanine. After removal of the gene trap of correctly targeted clones by transient expression of Cre-recombinase, clones expressing TetR-KRAB were established for conditional silencing of the HPRT locus.
(2) RNA interference (RNAi) is a widely used method for analysis of gene function. We have established a lentivirus-based, conditional shRNA expression system, which can be easily adapted for any target-gene by Gateway cloning. Here we describe a novel vector for the creation of conditional RNAi cell lines. This vector contains two gene expression units, using a conditional tetracycline-responsive promoter for an RNAi-inducing shRNA and a constitutive promoter for expression of TetR-T2A-GFP. Upon infection, expression of TetR prevents expression of the shRNA, which can be induced by blocking TetR function using doxycyline. By targeting the essential mitotic gene CDC27, we could demonstrate that this system is tight enough to allow the establishment of stable conditional RNAi cell lines to study the function of essential genes.

## 75. ER Stress Transkriptionsfaktor XBP1 reguliert NFκB-Aktivierung in Darmepithelzellen

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**Hintergrund:** Der IRE1/XBP1 Signalweg stellt bei der Bewältigung von durch missgefaltete Proteine auftretendem ER Stress einen zentralen Mechanismus dar. Die konditionelle Deletion von *Xbp1* spezifisch im intestinalen Epithel von Mäusen führte zur spontanen Enteritis, die jener bei chronisch entzündlichen Darmerkrankungen (CED) stark ähnelt, und Polymorphismen im *XBP1* Gen sind sowohl mit Morbus Crohn, als auch Colitis ulcerosa assoziiert. Zudem führte *Xbp1* Deletion im Darmepithel zur Panethzelldepletion aufgrund von Apoptose, sowie zu einem pro-inflammatorischen Phänotyp des Epithels mit vermehrter Phosphorylierung von JNK. Wir testeten die Hypothese, dass verminderte XBP1 Funktion zu verstärkter Aktivierung von NFκB führt, einem zentralen pro-inflammatorischen Signalweg.

**Methoden:** Die Darmepithelzellinie MODE-K wurde retroviral entweder nur für XBP1 gesilenced oder zusätzlich mittels siRNA für IRE1α oder IKK2, und die Zellen mit TLR Liganden oder TNFα stimuliert. Phosphorylierung von IKKs, IκBα und NFκB wurden mit phospho-spezifischen Antikörpern untersucht. DNA-Bindungsaktivität nukleärer Extrakte an die NFκB Konsensussequenz wurde geprüft und die Expression des klassischen Zielgens des kanonischen NFκB Signalwegs, IκBα mit qPCR gemessen.

**Ergebnisse:** TNFα Stimulation führte zu einer stärkeren und längeren IKK Phosphorylierung in MODE-K Zellen mit gesilenceter XBP1 Expression. IκBα als auch nukleäre NFκB Phosphorylierung nach TNFα Stimulation waren zu frühen Zeitpunkten im Vergleich zu Kontroll-gesilenceten MODE-K Zellen erhöht und NFκB wurde länger im Kern retiniert. Konsekutiv konnten wir vermehrte NFκB p65 Bindungsaktivität in nukleären Extrakten von XBP1-gesilenceten MODE-Ks nach TNFα Stimulation nachweisen. Entsprechend fanden wir vermehrte Expression des typischen NFκB Zielgenes IκBα in TNFα bzw. TLR3 Ligand-stimulierten MODE-K Zellen mit gesilenceter XBP1 Expression. Diese transkriptionelle Aktivierung war funktionell abhängig von IRE1α oder IKK2, wie anhand Doppel-Silencing Experimenten gezeigt werden konnte.

**Diskussion:** Wir zeigen, dass vermehrter ER Stress durch XBP1 knock-down in Darmepithelzellen zu einer deutlichen Überaktivierung des NFκB Signalwegs führt. Dies ist wahrscheinlich auf die Überaktivierung von IRE1α in XBP1-defizienten Darmepithelzellen zurückzuführen, nachdem Co-Silencing von IRE1α den Effekt aufheben konnte. Unsere Daten zeigen, dass der pro-inflammatorische Phänotyp, der als Konsequenz hypomorpher XBP1 Funktion auftritt, die Dysregulation des pro-inflammatorischen Schlüssel-Signaltransduktionswegs NFκB beinhaltet.

## 76. Premature Immunosenescence and a Disturbed Peripheral Naive T-cell Homeostasis in Children with Juvenile Idiopathic Arthritis

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**Background:** Immunosenescence (immunological aging) is characterized by diminished thymic output of naive T-cells, compensatory peripheral proliferation of mature T-cells and oligoclonal expansions of specific CD28-negative T-cells. The study was aimed to evaluate markers of premature immunosenescence in children with juvenile idiopathic arthritis (JIA) (n=22) and the influence of latent cytomegalovirus (CMV) on CD28-expressing T-cell subpopulations and relative telomere length (RTL) compared to age-matched healthy donors (HD) (n=37). T-cell- receptor-excision-circles (TRECs) were analyzed as a marker of thymic function and peripheral replication of naive T-cells.
**Methods:** T cells were characterized by flowcytometry. RTL and TRECs were analyzed by PCR.
**Results:** JIA patients demonstrated an age-dependent decrease of $CD4^+CD45RA^+CD62L^+$ naive T-cells and an increase of $CD4^+CD45RO^+$ memory T-cells. JIA patients showed decreased TRECs compared to HD (p=0.002). Correlation of TRECs with age were present only in HD (p=0.0001). JIA patients showed shortened RTLs (p=0.01) and increased percentages of proliferating Ki67+ naive T-cells (p=0.001) correlating with disease duration (p=0.003). CMV-positive JIA patients did not show a markedly expansion of CD28-negative T-cells as known from CMV-positive HD. CMV was an independent factor for loss of CD28 regardless of age in HD, but not in JIA patients.
**Conclusion:** The present study supports the hypothesis that a disturbed peripheral T-cell homeostasis, also, in response to latent CMV infection, and signs of premature immunosenescence may be characteristic features of JIA patients. These alterations of peripheral T-cell proliferation may also play a role in the pathogenesis ofJIA.

## 77. Combined Loss of the BH3-only Proteins Bim and Bmf Impairs Developmental Cell Death and Causes Autoimmune Pathology-associated Premature Lethality in Mice

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Apoptosis or programmed cell death executes the decision of a certain cell to commit suicide in order to promote embryonic development or prevent pathogenesis in the adult organism. An intrinsic stress signal (i.e. DNA damage, ligation of antigen receptors, disruption of the cytoskeleton) leads to the activation of either one or a certain set of BH3-only proteins, such as Bim and Bmf. These proteins function by antagonizing their pro-survival counterparts belonging to the same protein family, i.e. Bcl-2 or Mcl-1, thereby initiating apoptosis.

The immune system belongs to the tissues with the highest cellular turnover where cell death has to balance the new formation of millions of cells per day to maintain tissue size. The removal of aged, damaged or potentially harmful lymphocytes is critical to prevent immunopathology, but the molecular players involved in this process are still poorly defined.

Loss of Bim promotes accumulation of B as well as T cells in mice and renders these cells more resistant towards a broad range of apoptotic stimuli. Bmf-dependent apoptosis is more restricted to the B cell compartment and its loss has been shown to promote lymphomagenesis in mice.

To investigate whether the function of Bim and Bmf overlap, we generated mice lacking both genes and compared their phenotype to that of the single-knockout and wild-type (wt) control mice.

Surprisingly, Bim and Bmf have overlapping and partially non-redundant functions during development, as demonstrated by the persistence of inter-digital webs, vaginal aplasia and malocclusion of the incisors - phenotypes that could not be observed in the single-knockout mice. Furthermore, both proteins contribute to the regulation of B cell homeostasis and lymphocyte apoptosis towards a plethora of apoptotic stimuli. Strikingly, the majority of bim-/-bmf-/- mice die prematurely due to the development of an SLE-like autoimmune disease, although maintained on a non-autoimmune prone genetic background (pure C57BL/6). Systemic lupus erythematosus (SLE) is a multifactorial disease that is primarily defined by increased levels of autoantibody-complexes that lead to organ destruction, mostly involving the kidneys. Consistently, we found accumulation and increased apoptosis resistance in immature, mature naive, as well as plasma B cells accompanied by overproduction of auto-antibodies and renal immune complex deposits. Furthermore, these mice have increased levels of activated T cells and dendritic cells show increased resistance to spontaneous apoptosis.

Collectively, our data demonstrates that the combined deficiency of the two BH3-only proteins Bim and Bmf, but not of either gene alone induces developmental phenotypes as well as a severe SLE-like phenotype on a highly autoimmune-resistant mouse genetic background.

## 78. The Turnover of Synovial T Cells in Persistent Oligoarticular Juvenile Idiopathic Arthritis is Higher than in T Cells in the Peripheral Blood

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**Introduction:** Juvenile idiopathic arthritis (JIA) summarizes a group of inflammatory diseases of childhood. The etiology remains still unclear. In JIA T cells have been demonstrated to play key roles in the pathogenesis. T-cell proliferation in JIA may be different in the peripheral blood (PB) and the synovial fluid (SF). The aim of this study is to demonstrate the turnover of T-cells in the PB and SF of patients with persistent oligoarticular JIA (oJIA) compared to controls.

**Patients and Methods:** Matched pairs of samples were investigated derived from PB and synovial fluid SF of 9 patients with persistent oJIA. The cells from PB and SF were determined by flow cytometry.

**Results:** The majority of the PBMC and IAMC were in phase G0/G1, with fewer than 1% in S phase. In the SF the percentage of cells in the S phase is higher than in the PB. The percentage of cells in the S phase in SF is equal to the result in the control group. In conclusion the turnover of synovial T- cells in persistent oJIA is higher than in the PB.

## 79. Role of gp130 in Neuroimmune Interactions – Overview of Current Research

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**Background:** gp130 is the common signal transducer subunit shared by the cytokines IL-6, CNTF, LIF, OSM, CT1 and others. These cytokines have pleiotropic actions and regulate neuroregeneration after nerve lesion and synaptic transmission at spinal cord level. In addition, they are important regulators of innate immunity and neuroinflammation. We currently investigate the role of neuronal gp130 signal transducer in the control of neuroimmune interactions in experimental models of inflammation, nerve lesion and malignant tumor.

**Methods:** We have used an interdisciplinary approach including gene expression analysis (qRT-PCR with Taqman® technology, Affymetrix gene chip), immunocytochemistry and in vivo lesion and inflammation models together with behavioural analysis (Hargreaves test, v. Frey mechanical testing). F4/80 staining has been used to detect macrophages and indirect immune fluorescence to stain for neuronal markers like the neuropeptide calcitonin-gene related peptide (CGRP) or the lectin IB4. For detection of microglia antibody againts OX-42 or Iba-1 are used. Immune stainings with specific markers are currently performed in order to detect other immune cells (granulocytes, T cells) in nerve lesions, inflamed tissue and experimental tumors in SNS-gp13$^{-/-}$ and gp130$^{fl/fl}$ control mice. Peripheral injury by so far unknown mechanisms induces activation of microglia in the central nervous system. We hypothesise that microglia activation may be largely mediated by gp130 signal transducer activation. Therefore, we analyse whether microglia activation is preserved in SNS-gp130$^{-/-}$ in comparison to gp130$^{fl/fl}$ control mice. Conditional gp130 null mutant mice are available that lack gp130 in other cell types. gp130 transduction strategies (Amaxa, viral constructs) will be used for rescue of phenotype. Last, neutralisation strategies for gp130 will be used.

**Results:** Mice lacking gp130 selectively in pain sensing primary afferent neurons, the nociceptors, show reduced hypersensitivity to painful stimuli in the CFA inflammation model. Also, hypersensitivity as a consequence of nerve injury is attenuated. In a pilot study, we have analysed the nerves at the lesion side. In mice lacking the neuronal g130 signal transducer we found reduced CCL2 chemokine mRNA and protein expression in the vicinity of the lesion which was accompanied by a reduced number of F4/80 positive cells, presumably macrophages.

**Conclusion:** Our data suggest that signals initiated by gp130 expressing injured and adjacent non-injured neurons regulate the invasion of macrophages into lesion sites which then secrete elements that signal back to neurons. Our data support an important role of neuronal gp130 for neuroimmune interactions in peripheral tissues. We expect to fully elucidate the role of gp130 in the regulation of neuroimmune interactions.

## 80. The Effect of Age on the Immunmodulatory Impact of Human Mesenchymal Stem Cells

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Several studies have shown the immunosuppressive effect of bone marrow mesenchymal stem cells (MSC) on lymphocytes. However, little is known about this interaction in elderly persons. We hypothesized that the immunregulative capacity of MSC is reduced in later life.

Therefore we analyzed the effect of human MSC from young and old donors on the activation and proliferation of human peripheral blood mononuclear cells (PBMC) from young (<30 years) and elderly donors (> 65 years) in a coculture system. MSC were isolated from the iliac crest of healthy donors by collagenase digestion and Ficoll gradient centrifugation and subsequent culture in 3% O2 and 5% CO2. PBMC were cultured with MSC at a ratio of 10:1 in a cell to cell contact coculture and analyzed at different time points. Using flow cytometric analyses of CFSE labeled PBMC we demonstrated that the phytohaemaglutinin (PHA) induced proliferation of these cells is suppressed in coculture with MSC independently of the age of the donors of either MSC or PBMC. Despite the fact that proliferation was suppressed, CD8 and CD4 T-cells expressed the early activation marker CD69 after 24h treatment with PHA and produce IFN-$\gamma$ independently of the presence of young or old MSC. Using ELISAs we also investigated the levels of interleukin 6 in the culture supernatant after 24h and 48h.

IL6 was produced by MSC on its own in both age groups, but was strongly increased following coculture, demonstrating intact MSC function in elderly donors. In conclusion we could show that the immunregulatory effect of MSC is conserved in old age. These findings are clinical relevant, as they demonstrate that MSC do not lose their potential for achieving transplantation tolerance in elderly persons.

## 81. Regulation of Iron Homeostasis in Anemia of Chronic Disease and Iron Deficiency Anemia - Diagnostic and Therapeutic Implications

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**Introduction:** The anemia of chronic disease (ACD) is characterized by macrophage iron retention induced by cytokines and the master regulator hepcidin. Hepcidin controls cellular iron efflux upon binding to the iron export protein ferroportin. Many patients, however, present with both, ACD and iron deficiency anemia (ACD/IDA), the latter due to chronic blood loss.

**Methods:** We used a rat model of ACD due to chronic arthritis and mimicked ACD/IDA by additional phlebotomy in order to define differing iron regulatory pathways.

**Results:** Iron retention during inflammation occurs in macrophages and the spleen, but not in the liver. In rats and humans suffering from ACD, serum hepcidin concentrations are elevated, which is paralleled by reduced duodenal and macrophage expression of ferroportin. Individuals suffering from ACD/IDA have significantly lower hepcidin levels than ACD subjects, and ACD/IDA individuals, in contrast to ACD subjects, were able to absorb dietary iron from the gut and to mobilize iron from macrophages.

**Conclusion:** Circulating hepcidin levels affect iron traffic in ACD and ACD/IDA and are more responsive to the erythropoietic demands for iron than to inflammation. Hepcidin determination may aid to differentiate between ACD and ACD/IDA and in selecting appropriate therapy for these patients.

## 82. *Staphylococcus Aureus* Lipoproteins Affect Immune Response and Body Iron Homeostasis

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**Background:** Toll-like receptor 2 (TLR2) is a receptor for lipoproteins (lpp) and signals by the adaptor MyD88. Interactions of TLRs with lpps are known to be crucial for eliciting an adequate host response against bacterial infections. As the impact of lpp on TLR2-MyD88 activation for *S. aureus* in systemic infection is unknown, we studied the effects of lpp maturation in two *S. aureus* strains, SA113 and Newman, deficient in the enzyme prolipoprotein diacylglyceryl transferase (Δ*lgt*).

**Methods:** C57BL/6 mice were injected either 1 x $10^6$ to 1 x $10^8$ cfu of *S. aureus* wt or *S. aureus* Δlgt (dissolved in 200 μL of 0.9% NaCl) or 0.9% NaCl into the lateral tail vein. The animals were sacrificed 24 hours or 7 days after initiation of the infection. We extracted RNA and protein from spleen and liver and studied the expression of critical genes in iron homeostasis and innate immunity by means of QRT-PCR and Western blots. Iron content of organs was quantified by a colorimetric assay.

**Results:** The injection of *Staphylococcus aureus* resulted in a severe sepsis with a stimulation of pro-inflammatory and anti-inflammatory cytokine formation. Δ*lgt* staphylococcal infection resulted in impaired immune response, reflected by a decreased expression of the pro-inflammatory cytokine TNF-α as compared to wt staphylococcal infection, along with a modulation in the expression of iron homeostasis genes in the liver and most pronounced in the spleen which was most evident when investigating the expression of the iron export protein ferroportin and divalent metal transporter-1. We further observed significant differences of iron content in organs between *S. aureus* wt infected mice and animals exposed to *S. aureus* Δ*lgt*.

**Conclusions:** These results indicate that lpp induction of inflammatory cytokines has an impact on iron transport mechanisms. Changes in iron metabolism are known to influence host immune effector functions as well as bacterial growth rates and survival and may therefore contribute to the disease severity in *S. aureus* sepsis.

## 83. HSP60 T Cell Epitopes and Anti-HSP60 Autoantibody are Involved in the Initiation and Progression of Atherosclerosis

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Atherosclerosis is a multifactorial chronic, inflammatory disease characterized by the appearance of intralesional T cells, macrophages, and dendritic cells. These cells contribute to the inflammatory process in the arterial wall by mechanisms that are not yet completely elucidated. Surface expression of HSP60 by arterial endothelial cells is believed to be a hallmark of the earliest stages of atherosclerosis. The induction of HSP60 and adhesion molecules is increased in endothelial cells subjected to classical atherosclerosis risk factors. These HSP60 positive endothelial cells can then become a target for pre-existent innate and adaptive cellular and/or humoral immunity. To characterize the proatherogenic HSP60 epitope/s leading to early atherosclerosis could therefore lead to new therapeutic interventions targeting the actual disease process in the arterial wall.

The aim of this study was to phenotypically and functionally characterize T cells isolated from early atherosclerotic lesions and to determine the potential reactivity to HSP60.

Iliac arteries and peripheral blood were collected from transplantation organ donors (n=6) and analyzed by immunohistochemistry and flow cytometry. The T cell immune-response was determined by proliferation assays against total recombinant human HSP60 protein (hHSP60) and HSP60 15mer overlapping peptides thereof.

Immunohistological analysis demonstrated the presence of $CD4^+$, $CD8^+$, dendritic cells ($CD1a^+$), and macrophages ($CD68^+$) within the intima in early atherosclerotic lesions. Surface expression of HSP60 was detected in endothelial cells and HLA class II cells. The predominant phenotype of intralesional T cells was defined as $CD4^+CD45RO^+CD28^+CD25^+FoxP3^+$ cells. Both, $CD4^+$ and $CD8^+$ T cells showed an increased production of IFN-$\gamma$ compared to IL-10 and TGF-$\beta$. Interestingly, a considerable percentage of $CD4^+$ T cells still produced IL-4 as well as IL-17. In vitro, isolated intra-lesional early atherosclerotic T cells proliferate in response to whole hHSP60 protein and derived peptides suggesting the presence of HSP60 atherogenic epitopes. Significantly increased levels of circulating anti-HSP60 autoantibodies were found in sera of patients with early lesions and even more pronounced in patients with late lesions as compared to normal donors.

In conclusion, this is the first demonstration in early, clinically still inapparent human atherosclerotic lesions that proves our previously proposed concept that HSP60 reactive T cells initiate the early inflammatory stage of atherosclerosis by recognition of atherogenic HSP60 epitopes and the anti-HSP60 antibodies accelerate and perpetuate the disease. These epitopes may be an attractive diagnostic and therapeutic target for atherosclerosis.

## 84. Differential Effects of Immunosuppressants in Cultured Renal Fibroblasts and Epithelial Proximal Tubule Cells. A Role in Posttransplant Fibrogenesis?

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**Introduction**: While the use of immunosuppressants is mandatory for the long-term survival of allograft organs, common immunosuppressive agents like the calcineurin inhibitors cyclosporin A (CsA) and tacrolimus (FK506) have serious nephrotoxic potential. Drugs which act on different pathways, like the mTOR inhibitor rapamycin (sirolimus) have therefore been introduced to lessen nephrotoxic adverse effects. Here we compare the effects of three important immunosuppressants on cell proliferation and senescence in cultured renal epithelial and mesenchymal cell lines.

**Methods:** The renal cell lines HK-2, RPTEC-TERT1 (both epithelial cells from proximal tubule), and TK-173 (fibroblasts) were grown to confluence. CsA, FK506, and sirolimus were then added to serum-free culture medium for 24 hours at maximum concentrations of 10 µM. Cell proliferation status was determined by cell number (resazurin conversion), DNA synthesis (BrdU incorporation), and expression levels of the cell cycle inhibitors p16 and p21 (real-time quantitative PCR). Oxidative stress was monitored by $H_2O_2$ production (Amplex Red conversion).

**Results:** Both proximal tubule cell lines showed clear signs of oxidative stress (increased $H_2O_2$ production) after application of CsA. DNA synthesis was reduced about 50% by CsA in epithelial cells. Markedly smaller effects were observed under FK506 and sirolimus treatment. In RPTEC-TERT1 cells, the expression of the senescence marker p21 was induced 2,5-fold by 10 µM CsA, but not by the other immunosuppressants. p16 expression was not affected under any condition. In fibroblasts, none of the drugs tested induced oxidative stress. In contrast to the epithelial cell lines, fibroblast growth was impaired most by FK506, where the growth-inhibited fibroblasts did not show upregulation of p21. Sirolimus in general had no pronounced effects on renal cells, apart from a slight growth inhibition. **Conclusions:** Posttransplantative complications like chronic renal allograft dysfunction may be partially induced by senescence of epithelial cells and a concomitant overgrowth of residential fibroblasts. Our data indicate a differential effect of cyclosporin A and tacrolimus on the proliferation of renal epithelial cells and renal fibroblasts in vitro. While cyclosporin A induced oxidative stress and senescence-like growth arrest in epithelial cells, fibroblast growth was impaired most by tacrolimus, but without an increase in the expression of senescence markers. This may in part contribute to the lower fibrogenic potential of tacrolimus.

## 85. The Effect of Erythropoietin in a Rat Model of the Anemia of Chronic Disease

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**Background:** The anemia of chronic disease (ACD) is the most important aneamia of hospitalized patients and one of the most common anemias in the total population. It is found in various chronic disorders, primarily inflammatory diseases and cancer. Improving the aneamia would be desireable in many cases, as these patients suffer from a reduced quality of life. However, treatment results with iron or erythropoetin are often unsattisfactory.

**Methods:** We used a rat model of ACD, where bacterial peptidoglycanes cause a chronic inflammation, mainly arthritis. This is, to the best of our knowledge, the only inflammatory rodent model causing a chronic anemia and allowed us to test the effect of erythropoietin on iron parameters, importantly also the expression of hepcidin, a small peptide today known as the central regulator of iron metabolism. Hepcidin inhibits the only known cellular iron exporter ferroportin.

**Results:** We could demonstrate a long term and short term effect of erythropoietin treatment on serum hepcidin. However, in the ACD there is not necessariliy and immediate effect of erythropoietin on the anaemia. Erythropoietin can reduce serum hepcidin after days already, but red blood parameters follow not before the inflammatory activity decreases. We demonstrated a correlation between the serum hepcidin levels when starting eryhtropoietin therapy and the change on hemoglobin levels, as higher hepcidin predicts a worse response to therapy. Erythropoietin reduced ferritin in liver and spleen, thereby enabling the activation of iron from these storage sites. Accordingly, the iron importer DMT1 was decreased in the liver, while the iron exporter ferroportin increased in the liver and the spleen.

**Conclusion:** Our results will have future implications on the erythropoietin therapy of the ACD. Serum hepcidin levels may be a prognostic factor of the therapeutic effect, and may have to be considered when planing dosage of erythropoietin and treatment duration.

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