

GITR-GITRL interactions modulate IgG4 induction by regulatory T cells in concert with CTLA-4, TGF-beta and IL-10

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Regulatory T cells are an important subset of T cells that have been intensively studied during the last decade. In chronic onchocerciasis, IL-10 producing antigen specific T regulatory-1 cells (Tr1) could be cloned in high frequency, in particular from patients having high worm load and little pathology. The mechanisms proposed to explain Tr-cell immunosuppressive activities include IL-10, TGF-beta, CTLA-4, and GITR. It has been recently shown that IL-10 produced by Tr1 cells induces B-cells to secrete IgG4, a non-inflammatory antibody, in a cell-contact-dependent manner. The present study was aimed at understanding the mechanism whereby Tr1 cells preferentially induce IgG4. For this purpose, we generated FOXP3⁺GITR⁺IL10⁺ Tr1 clones from human PBMC, using vitamin D3, dexamethason and tetanus toxoid as antigen model. These Tr1 cells were co-cultured with autologous purified B-cells to induce IgG4. Using blocking antibodies, we found that neutralizing anti-GITR antibody selectively prevented IgG4 production and increased IgG2 production. Antibodies to GITRL, IL-10, CTLA-4 and to TGF-beta also blocked IgG4 production, while anti-ICOS antibody had no effect on IgG4 production. Interestingly, the production of IL-10 by the co-cultured cells increased in the presence of anti-GITR antibodies. Hence, the blocking of IgG4 induction by anti-GITR antibodies was reversed by the addition of excess rIL-10 but not of rTGF-beta. These results show that GITR-GITRL interactions modulate IgG4 induction partially through induction of other factors chiefly IL10.

T cell activation by *Leishmania major* lysate in the draining lymph node of BALB/c mice

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Recently it was shown that the immune response against experimental *Leishmania major* (*L.m.*) parasites is induced very early (24h) in the draining lymph node by resident dendritic cells. It is assumed that soluble antigens, released by live, damaged or killed *L.m.* contribute to this early activation of resident dendritic cells. In order to reveal the impact of these soluble factors we prepared lysates of *L.m.* and compared the expression of cytokine mRNAs and rate of T cell proliferation to that of conventional *L.m.* preparations (2×10^6) after inoculation in BALB/c mice. In order to increase the sensitivity we established a combination of laser microdissection and real-time RT-PCR. Our results show that *L.m.* lysates induce cytokine mRNAs after 24h at a similar level than experimental *L.m.*, but additionally increase most of them already after 8h (IL-1b, IL-2, IL-4, IL-27p28, Cxcl-10). Interestingly, the main difference between these two inoculations could be found in the expression of IL-4 and IFN-gamma mRNA. Furthermore, although *L.m.* lysates do not induce clinical disease, they are sufficient to activate strong T cell proliferation within 72h in the T cell zone of lymph nodes that resembles the proliferation pattern of conventional experimental *L.m.* infections.

Taken together our data indicate that the early T cell activation is initiated irrespective of whether leishmania or low-molecular weight fragments are injected. Further experiments using viable *L.m.* devoid of any soluble antigens and cell debris will reveal the impact of the early immune response (cytokine expression, T cell activation) on the course of disease.

IFIT-2 – a putative novel negative regulator of proinflammatory responses

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Interferon-induced tetratricopeptide repeat protein (IFIT-) 2 is induced upon acute infection with *Yersinia enterocolitica* in CD11b-positive cells of the spleen of mice as well as in the colon of IL-2 deficient mice which develop inflammatory bowel disease. IFIT-2 is known to be highly induced by LPS, viral dsRNA or interferons. Recently it was reported that mouse IFIT2 (P54) affects protein synthesis by interaction with the translation initiation factor eIF3c. Therefore we speculated that this gene could represent a negative regulator of host responses by down regulating protein synthesis. To address the role of IFIT2, stably transfected RAW 264.7 macrophages were established overexpressing IFIT-2. These cells were viable and showed similar proliferation as control cells. IFIT-2 overexpression did not alter LPS triggered p38, ERK, JNK and I-kappaB phosphorylation indicating that IFIT-2 does not affect LPS mediated signal transduction. However, overexpression of IFIT-2 in RAW 264.7 macrophages reduced LPS induced TNF-alpha, IL-6 and MIP-2 secretion by more than 80%. In contrast, LPS induced expression of IFIT-1, early growth response 1 or tristetraprolin was not affected by IFIT-2 overexpression, leading to the suggestion that IFIT-2 affects protein expression in a very selective manner. Thus, IFIT-2 may represent a novel negative regulator of proinflammatory responses triggered by bacterial components which acts on a post-transcriptional level and which could be in concert with other mechanisms involved in the regulation of inflammatory responses.

MURINE CYTOMEGALOVIRUS vRAPs PREVENT ANTIGEN PRESENTATION IN HOST TISSUES BUT HAVE LITTLE IMPACT ON CD8 T CELL PRIMING

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Cytomegaloviruses code for vRAPs, viral regulators of antigen presentation. Murine cytomegalovirus (mCMV) expresses three glycoproteins that interfere with the trafficking of MHC class I molecules: the negative vRAP m152/gp40 retains peptide-loaded MHC class-I complexes in a cis-Golgi compartment, whereas the positive vRAP m04/gp34 antagonizes m152/gp40 by transporting MHC class I complexes to the cell surface. Finally, the negative vRAP m06/gp48 antagonizes m04/gp34 by routing class I complexes to the lysosome for degradation.

Notably, dose-response curves for priming revealed that optimal CD8 T cell priming can be obtained with a virus dose of just 10^3 PFU, but with no difference between a vRAP triple-deletion mutant and the WT virus. This indicates that vRAPs have little effect on the priming of an immune response.

In contrast, in a model of immunotherapy by adoptive transfer of CD8 T cells primed with either mutant or WT virus, protection of transfer recipients against virus replication in vital organs was found to be more efficient after infection with the mutant. These data show that the main role of vRAPs is to prevent antigen presentation in host tissues during the antiviral effector phase.

Enduring arrest of TLR2 in the ER abrogates TLR2 surface expression and dependent cell activation specifically

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Toll-like receptor (TLR) signaling aims at invader clearance and/or reconstitution of regular homeostasis but might lead to adverse effects if triggered extensively: incessant TLR agonist challenge induces and maintains chronic inflammation while overactivation in the course of acute infection causes septic shock. Thus, inhibition of pattern recognition receptor function of TLRs might provide benefit. While cell surface TLRs are blocked transiently for instance by binding of antagonistic antibodies, targeting of endosomal TLR ectodomains would require uptake or diffusion of a respective agent into the cell. Inhibition of intracellular TLR transport is a different strategy for enduring prevention of TLR mediated cell activation. In order to validate this statement, we cloned the variable domains of a crossreactive anti TLR2 mAb for expression of a single chain minibody fused to a peptide mediating ER retention. Intrabody (Ib) plasmid transfection into human fibroblasts or murine macrophages abrogated TLR2 cell surface expression. Importantly, this effect was specific. Furthermore, also cell activation upon TLR2 specific challenge was inhibited. In order to enlarge the time window of effectiveness and for in vivo applicability, an Ib-adenovirus variant was prepared. Comparative infection of cells using a control adenovirus showed high effectiveness of transformation, as well as prolonged and specific inhibition of TLR2 function. Also since localization of TLR9 is endosomal, we generated a TLR9 specific Ib. We will report on progress of TLR2 specific Ib-adenovirus application in vivo, as well as an attempt to arrest TLR9 in the ER in vitro.

Hydrophobic antigens of *Mycobacterium tuberculosis* prime naive human T-lymphocytes

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Tuberculosis is continuing to be a major threat to mankind, due to the increasing incidence of HIV and the emergence of untreatable strains. Even though BCG-vaccination prevents severe forms of tuberculosis in childhood it fails to protect from primary infection in adults and reactivation of latent tuberculosis. Therefore novel vaccines are desperately needed to combat the spread of tuberculosis. Because the cell wall of *Mycobacterium tuberculosis* (*MTb*), the causative agent of tuberculosis, contains many hydrophobic molecules, we tested their ability to trigger T cell responses in human T-lymphocytes. We used a chloroform-methanol extract of *MTb* (MTb-CME) as a source for hydrophobic antigens. Initial experiments using MTb-CME pulsed dendritic cells and autologous peripheral blood mononuclear cells demonstrated that hydrophobic antigens induce proliferation of T-lymphocytes independent of adjuvant activity mediated by Toll-like-receptors. Precondition for every successful vaccination is the capability of the antigen to activate naive T-cells. Therefore we isolated naive T-lymphocytes expressing CD45RA and chemokine receptor 7 by cell sorting. We detected significant proliferation of naive T-Lymphocytes after 6 days using autologous mature dendritic cells as antigen-presenting cells. Activated naive T-lymphocytes produced IFN-gamma but no TNF-alpha or interleukin-4. These results demonstrate that hydrophobic antigens of *MTb* prime naive T-cells and are therefore attractive molecules to be further evaluated as vaccine candidates. Ongoing experiments are designed to investigate the cytolytic and antimicrobial activity of activated naive T-lymphocytes and to identify the immunogenic molecules within MTb-CME.

Mechanismen der erhöhten Tuberkulose-Inzidenz unter Therapie mit Tumor-Nekrose-Faktor Antikörpern.

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Bei der Pathophysiologie von Autoimmunerkrankungen spielt die Freisetzung entzündlicher Mediatoren – allen voran der Tumor-Nekrose-Faktor (TNF) – eine entscheidende Rolle. So ist bei der rheumatoiden Arthritis (RA) TNF entscheidend an der Zerstörung des Gelenkknorpels beteiligt. TNF ist jedoch auch essentiell bei der Immunantwort gegen die Tuberkulose. Dies erklärt das Auftreten von Tuberkuloseerkrankungen als Nebenwirkung einer anti-TNF Therapie. Ziel dieses Projektes war die Aufdeckung der Mechanismen dieser Beobachtung. Dazu wurden von RA Patienten vor und nach Beginn der Therapie mit Infliximab Blutproben entnommen und Parameter untersucht, die für die Immunantwort bei der Tuberkulose entscheidend sind. Unsere Experimente zeigten, dass NK-Zellen und CD8⁺ T-Zellen (ZTL) nach der ersten Infusion signifikant abnahmen. Als möglicher Mechanismus dieser Abnahme wurden Antikörper- und Komplementabhängige Zellyse untersucht. Zunächst inkubierten wir PBMC von gesunden Spendern oder Patienten mit aktiver RA mit anti-TNF-Antikörpern und detektierten apoptotische Zellen mittels Annexin. Es zeigte sich, dass Infliximab selektiv in den aktivierten Zellen von RA Patienten Apoptose induziert. Um den Beitrag von Komplementvermittelter Zellyse zu untersuchen, etablierten wir ein Versuchssystem, das es erlaubt CD8/CD56 positive Zellen, die membranständiges TNF exprimieren und Infliximab binden, zu detektieren. Dazu inkubierten wir PBMC von RA Patienten mit Biotin markiertem Infliximab. In Anwesenheit von Kaninchen-Komplement nahm die Anzahl der ZTL in allen vier untersuchten Spendern um bis zu 80% ab.

Somit sind sowohl die Antikörper- wie auch Komplementvermittelte Zellyse Mechanismen für die Abnahme der Granulysin exprimierender ZTL bei RA Patienten unter Infliximab Therapie.

Diese Befunde deuten auf eine wichtige Rolle von Granulysin bei der Immunantwort gegen die Tuberkulose hin.

Doxycycline Reduces Plasma VEGF-C/sVEGFR-3 and Improves Pathology in Lymphatic Filariasis

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Lymphatic filariasis is a disease of considerable socioeconomic burden in the tropics. Presently used antifilarial drugs are able to strongly reduce transmission and will thus ultimately lower the burden of morbidity associated with the infection, however, a chemotherapeutic principle that directly induces a halt or improvement in the progression of the morbidity in already infected individuals would constitute a major lead. In search of such drug to complement the existing ones, we performed a double-blind, placebo-controlled trial of a 6-week regimen of 200 mg/day doxycycline in an area endemic for bancroftian filariasis in Ghana. *Wolbachia* load, microfilaremia, antigenemia, and frequency of filarial dance sign (FDS) were significantly reduced in the doxycycline group compared to the placebo group. The extent of disease in lymphedema significantly improved following doxycycline, with the mean stage of lymphedema in the doxycycline-treated patients being significantly lower compared to placebo patients 12 month after treatment. The reduction in the stages manifested as better skin texture, a reduction of deep folds, and fewer deep skin folds. Furthermore, the dilation of supratesticular lymphatic vessels in doxycycline-treated patients was reduced significantly at 24 month post treatment, whereas there was no improvement in the placebo group. Preceding clinical improvement, the plasma levels of VEGF-C and sVEGFR-3 decreased significantly in the doxycycline treated patients to a level close to that of endemic normals, whereas there was no significant reduction in the placebo patients.

In conclusion, a 6-week regimen of antifilarial treatment with doxycycline against *Wuchereria bancrofti* showed strong macrofilaricidal activity and reduction in lymphangiogenic factors VEGF-C and sVEGFR-3, the latter being associated with amelioration of supratesticular dilated lymphatic vessels and with an improvement of pathology in lymphatic filariasis patients.

STAT3 - TLR2 interaction and rapid STAT3 phosphorylation upon TLR2 specific challenge

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Signal transducer and activator of transcription (STAT) 3 is a global negative-feedback regulator of inflammatory immune responses. Toll-like receptors (TLRs) mediate cellular recognition of microbial and viral products by binding and signal transduction through the cell membrane. Cytoplasmic interaction of TLRs with intracellular proteins initiates immune activation and inflammation. By analyzing proteins for interactions with the intracellular domain of TLR1 upon overexpression in yeast, we identified distinct candidate molecules. We analyzed interaction of endogenous or overexpressed proteins by immune precipitation from lysates of macrophages or fibroblasts, respectively. Our findings implicate recruitment of STAT3 to the intracellular domain of TLR2 in the course of TLR ligand induced cell activation. Furthermore, the adaptor molecule MyD88 interacted with STAT3 as well. Analysis of primary macrophages indicated TLR-dependent phosphorylation of STAT3 rapidly upon specific agonist administration. Differential dependence of STAT3 phosphorylation on expression of IL-1/TLR signaling molecules MyD88 or TRIF was evident from comparative analysis of cells challenged with different TLR agonists. Surprisingly, our results did not imply a role of IRAK1 in TLR induced STAT3 phosphorylation. However, application of different kinase inhibitors to cells challenged with TLR agonists indicated involvement of specific MAP kinases in possibly direct STAT3 phosphorylation observed. Comparative mRNA expression profiling of wild-type and STAT3 deficient macrophages challenged with the TLR2 agonist lipopeptide for different time periods identified differentially regulated genes. Our results implicate rapid STAT3 recruitment to activated TLRs and STAT3 phosphorylation in immune regulation.

In vitro effects of CD40L multimers on human and rhesus macaque PBMC subsets and monocyte-derived dendritic cells

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Novel adjuvants for the induction of cellular and humoral immune responses to pathogens such as HIV are needed and the rhesus macaque model provides the opportunity to test HIV vaccine candidates in vivo. CD40L (CD154) is physiologically expressed on activated T cells and acts as a potent stimulator of antigen presenting cells and could thus be useful for the priming of T cells for antigen-specific immune responses.

We therefore explored the effects of soluble dimerized trimers of human and rhesus macaque CD40L (MegaCD40L^{hu/rh}) on select subsets of primate PBMCs as well as monocyte-derived dendritic cells (DCs). When freshly isolated PBMCs were stimulated for 48 h with MegaCD40L myeloid DCs and B cells were activated, as demonstrated by up-regulation of surface markers of maturation. To further characterize effects on single leukocyte subsets, monocytes and B cells were magnetically separated from PBMCs and then stimulated for 48 h with MegaCD40L. Monocyte activation was demonstrated by the secretion of TNF while purified B cells proliferated and up-regulated surface markers of maturation. MegaCD40L also activated monocyte-derived DCs as determined by up-regulated surface markers of maturation and enhanced secretion of IL-12. These effects were blocked by co-incubation of the cells with a neutralizing anti-CD154 monoclonal antibody, underlining the specificity of the effect of MegaCD40L.

Further investigation of MegaCD40L effects on the interactions of antigen presenting cells with T cells is ongoing and is currently being extended to adenovirally expressed MegaCD40L.

Differential role of CCR2 on Gr1^{HI} and Gr1^{LO} monocyte migration in response to bacterial infection

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Monocytes are crucial immune effectors in bacterial infection, but the mechanisms governing their migration are not completely understood. The chemokine receptor 2 (CCR2) regulates migration of monocytes, which is important for the defense against several pathogens. It has been shown recently that CCR2 is required for bone marrow (BM) emigration of monocyte precursors expressing high levels of the Gr1 (Gr1^{HI}) molecules. A differential effect of CCR2 on the migration of Gr1^{HI} and Gr1^{LO} monocytes in response to bacterial infection remains to be clarified.

We have established a murine model of urinary tract infection (UTI) by injection of uropathogenic *E.coli* (UPEC) into the bladder of C57/BL6 mice. The numbers of macrophages in infected bladders of CCR2-deficient mice were reduced significantly. Using a recently published monocyte tracking method, we found that CCR2 was dispensable for immigration of both Gr1^{LO} and Gr1^{HI} monocytes into the infected bladder. Analysis of the blood of CCR2-deficient mice revealed a striking reduction of circulating monocytes, whereas no changes were seen in the BM. Mixed BM-chimeric mice, reconstituted with CD45.1 CCR2-competent and CD45.2 CCR2-deficient BM showed, that expression of CCR2 did not affect monocyte numbers in the BM, whereas the number of CD45.2 CCR2-deficient donor cells in the blood were severely decreased. These data indicated that CCR2 mediated emigration of monocyte precursors out of the BM, but neither immigration of mature monocytes into the BM, nor into the infected bladder in UTI.

Transcriptional analysis of proteins involved in peptidoglycan biosynthesis in *Chlamydomonas reinhardtii*

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The cytoplasmatic NOD proteins have been suggested as immune sensors for *C. pneumoniae* (*C.p.*). Muropeptides, small components of the bacterial peptidoglycan (PGN) represent the natural ligands of NOD, but could so far not be detected in *C.p.*. To investigate, whether enzymes involved in the synthesis and degradation of PGN are transcribed during the chlamydial infection cycle, we have followed their mRNA levels by transcriptional analysis. Therefore, primers for eleven specific PGN-enzymes i.e. *murA* to *murG*, *mraY*, *pbp2*, *amiA* and *nlpD*, as well as for three control genes (*lcrH2*, *omcB* and *groEL*) with known transcription patterns were designed. Chlamydial DNA and RNA were prepared from samples of two independent *C.p.* infection kinetics taken at nine different time points and were investigated by highly specific and sensitive real-time PCR. For all chosen genes specific RNA transcripts could be detected, which were quantified after normalizing to the respective *C.p.* number. The transcription pattern of all three control genes were in line with published results. For the genes involved in the synthesis and degradation of PGN a bell-shaped transcription curve was obtained, excepting *murE* and *mraY*, which was biphasically induced. The PGN synthesising enzymes showed maximal transcription between 24 and 36 hpi, while for the PGN degrading enzymes a maximum at 36 hpi was detected.

The fact, that all analysed PGN-relevant genes are transcribed, indicates that PGN most probably is synthesised in chlamydia. Ongoing work addresses the direct detection of muramic acid by MS analysis.

Adenovirus entry induces strong early production of type I IFN from splenic myeloid DC *in vivo* resulting in LPS hypersensitivity

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The production of type I IFN by adenoviruses (Ads) is poorly characterized. Previously we have shown that human adenovirus (Ad) infection sensitizes mice to bacterial endotoxin. We present data that this phenomenon is mediated by type I IFN since mice deficient for IFN alpha/beta-receptor do not develop LPS hypersensitivity after Ad treatment. Type I IFNs were found to be strongly induced as early as 4 hrs after infection with Ads. Experiments with UV inactivated and defective Ads showed that viral gene expression is not necessary for the induction of IFN alpha/beta. However, the absence of viral gene expression modified the kinetics and magnitude of IFN production. Toll like receptors (TLRs) 3, 7 and 9, recruited to endosomes play major roles in the innate immune recognition of viruses and subsequent IFN production. Experiments with mice deficient for TLRs 2,4 and 9, as well as for Myd88 and Trif ruled out any involvement of the TLR system. Furthermore, Ad endosomal escape was required for the induction of IFN alpha/beta. Ad induced IFN production was strongly dependent on positive feedback by IFN alpha/beta. Type I IFN production absolutely required IRF-7; however the role of IRF-3 *in vivo* was insignificant. Most of Ad induced type I IFN was synthesized in the spleen. *Ex-vivo* FACS-Sorting experiments showed that in contrast to other viruses, the majority of Ad-induced IFN alpha/beta was produced by splenic myeloid dendritic cells.

In vivo gene expression analysis of colitogenic *E. coli* mpk using differential fluorescence induction (DFI)

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DFI is a promoter-trapping technique that utilizes the green fluorescent protein (GFP) as a selectable marker to monitor promoter activity. In combination with fluorescence activated cell sorting (FACS), DFI allows high-throughput screening of gene expression in microorganisms.

We used the plasmid pANT-3 as vector for the promoterless *gfp* gene to construct a promoter-trap library of colitogenic *E. coli* mpk. For this purpose DNA fragments with length between 200 and 500 bp from the chromosomal genome of *E. coli* mpk were directly cloned in the vector upstream of the promoterless *gfp* gene. This technique allows identification of *E. coli* mpk promoter genes that are specifically induced in complex environments. The specificity of gene expression under certain environmental conditions was validated by induction of pH stress and heat shock in vitro.

Decreasing of pH and increase of temperature in *E. coli* mpk cultures led to upregulation of genes responsible for adaption to environmental stress responses. Furthermore gene expression of *E. coli* mpk in coculture with non-colitogenic *B. vulgatus* mpk was investigated and revealed an increase in the expression of *E. coli* mpk genes responsible for central metabolism.

The aim of this study is to analyse the gene expression of *E. coli* mpk induced during colonization of mice colonized with *E. coli* mpk, *B. vulgatus* mpk or both and thereby identifying putative colitogenic factors of *E. coli* mpk.

Role for toll-like receptor 4 and interleukin-12 receptor signaling in the susceptibility to allergic contact hypersensitivity

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Contact hypersensitivity (CHS) to reactive haptens is a T cell mediated inflammatory disease. The mechanisms of induction are yet unclear. Skin inflammation and allergen presentation by dendritic cells (DC) to naïve T cells are crucial events in the sensitization phase of CHS. Here interleukin(IL)-12 is considered to play an important role. The skin, as the site of exposure to contact allergens, is also continuously exposed to environmental microbial flora and thus to lipopolysaccharide(LPS) of Gram-negative bacteria, a highly potent inducer of inflammation. Here we analyzed the role of the LPS receptor toll-like receptor(TLR)4 and IL-12 receptor in the development of CHS. Using mice deficient in TLR4, IL-12Rbeta2 or both we show that TLR4 and IL-12 can mutually replace each other in the induction of CHS, indicating the existence of two distinct sensitization pathways. The concomitant absence of TLR4 and IL-12 receptor prevented DC-mediated generation of effector T cells and subsequent CHS response to 2,4,6-trinitro-1-chlorobenzene (TNCB), oxazolone and fluorescein isothiocyanate. Introduction of the TLR4 transgene into the TLR4/IL-12Rbeta2 mutant restored CHS inducibility, showing a requirement for TLR4 in IL-12-independent CHS induction. The induction of CHS proceeded also in germ-free mice, showing that an environmental microbial stimulus is not essential and that endogenous TLR4 ligand(s) probably suffice to initiate the TLR signalling required for CHS.

Induction of tolerogenic DC: an important role for IL-6

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Recently we showed that *E. coli* mpk inducing colitis in gnotobiotic IL-2^{-/-} mice and *B. vulgatus* mpk which does not induce colitis differentially modulate maturation of DC and their ability to prime CD4⁺ T cells. Infection of bone-marrow-derived-DC with *E. coli* induced a strong proinflammatory response, as well as maturation of DC and led to Th1 polarization. In contrast stimulation with *B. vulgatus* led to secretion of IL-6 only and differentiated DC to a semimature state, indicated by inhibition of Th1 responses and non-responsiveness towards subsequent stimulation by *E. coli*. The nonresponsiveness of *B. vulgatus* stimulated DC was abrogated by the addition of anti-IL-6 mAb or mimicked with rIL-6. This data suggests that *B. vulgatus*-induced IL-6 drives DC to a semimature state, thus non-responsive to proinflammatory activation by *E. coli*. We hypothesize that the differential modulation of DC responses upon stimulation with *E. coli* or *B. vulgatus* might be due to activation of different TLR. Stimulation of DC with TLR2 ligand induced the same cytokine pattern as stimulation with *B. vulgatus*, with low TNF-alpha secretion and high levels of IL-6 and differentiated DC into semimature state. Stimulation with TLR4 ligand revealed results comparable to stimulation with *E. coli* and led to maturation of DC. Furthermore we demonstrated that TLR2-tolerized DC developed cross-tolerance towards TLR4 and homotolerance towards TLR2 stimulation as TLR4-tolerized DC did, indicated by decreased TNF-alpha production. Induction of cross- or homotolerogenic DC by stimulation with low amounts of Pam3 or LPS proved to be IL-6 dependent, as the effect was abrogated in IL-6 deficient DC. However the dependency on IL-6 was most distinct in Pam3-treated DC developing cross tolerance towards TLR4 signalling. This data indicate that IL-6 among DC maturation might be the most relevant factor in induction of tolerogenic DC.

Mast cells initiate early anti-*Listeria* host defenses

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The Gram-positive bacterium *Listeria monocytogenes* (*L.m.*) is the etiological agent of Listeriosis. The early phase of this infection is characterized by a strong innate host response. This is emphasized by the fact that mice deficient in both T-cell and humoral immunity have a remarkable ability to control infection. Mast cells are among the principal effectors of innate immunity. Thus far, these cells have mainly been studied in the context of hyperreactive conditions such as allergy and autoimmune diseases. In the present study, we evaluated the significance of mast cells during the early phase of *L.m.* infection. Compared to controls, mice depleted of mast cell showed hundred-fold higher bacterial burden in multiple organs and were significantly impaired in the mobilization neutrophils. Although degranulation of mast cells was observed upon *L.m.* infection, bacteria were hardly found mainly in sucells but in neutrophils and macrophages. Thus, although not directly involved in bacterial uptake, mast cells control infection by initiating neutrophils influx to the site of infection. We show that this is in part attributable to the presynthesized TNF- α which is rapidly secreted via degranulation upon mast cell activation by *L.m.*

Effector immunity to aerogenic *Mycobacterium tuberculosis* infection requires MyD88 functions distinct from its role as an adaptor to Toll like receptors 2, 4 and 9

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The role of Toll-like receptors (TLR) and MyD88 for innate and adaptive immune responses to infection with *Mycobacterium tuberculosis* (*Mtb*) remains controversial. To address the potentially different impact of TLR-mediated pathogen recognition and MyD88-dependent signalling events on anti-mycobacterial protection we compared the outcome of *Mtb* infection in MyD88- and TLR2/4/9 triple-deficient mice. After aerosol infection, MyD88-deficient mice were able to produce pro-inflammatory cytokines albeit in a delayed fashion. This promoted the development of antigen-specific CD4⁺ T cells and the production of interferon-gamma to a similar extent as in wild-type mice. Moreover, MyD88-deficient mice were capable of expressing IFN-gamma-dependent inducible nitric oxide synthase and LRG-47 in infected lungs and efficiently produced reactive nitrogen intermediates. Although the induction of innate and adaptive immune responses was eventual comparable in *Mtb*-infected TLR2/4/9-deficient mice, only mice deficient for MyD88 rapidly succumbed to unrestrained mycobacterial growth, whereas TLR2/4/9-deficient mice controlled the replication of *Mtb*. Further analyses *in vitro* revealed that IFN-gamma stimulated phagosome maturation and restriction of mycobacterial growth were severely impaired only in *Mtb*-infected MyD88-deficient bone-marrow-derived macrophages. Our results demonstrate that after *Mtb* infection, neither TLRs 2, 4, 9 nor MyD88 are required for the induction and expression of adaptive T cell responses. Rather, MyD88 – but not TLR2, TLR4 and TLR9 – is critical for mustering macrophage effector mechanisms central to anti-mycobacterial protection.

STAPHYLOCOCCUS AUREUS CARRIERS NEUTRALIZE SUPERANTIGENS BY ANTIBODIES SPECIFIC FOR THEIR COLONIZING STRAIN

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Staphylococcus aureus carriers have a 4-fold increased risk for developing an *S. aureus* bacteremia under hospitalization, which is mostly caused by the colonizing strain. Paradoxically, the outcome is significantly better in carriers than in noncarriers. We hypothesized, that this is due to a tuning of the immune response by the colonizing strain, e.g. induction of immunity or tolerance. We have used SAGs as strain-specific indicator antigens to probe the humoral immune response of carriers and noncarriers. SAGs are secreted virulence factors with extraordinary variability in the species *S. aureus*, which induce massive unspecific T cell activation. We collected nose swabs and sera from 121 healthy blood donors. SAG genes were detected by multiplex-PCRs. Anti-SAG serum antibodies were determined in a proliferation assay using culture supernatants of the colonizing *S. aureus* strains. The SAG-neutralizing capacity of a serum from either a carrier or a noncarrier was determined as the reduction of proliferation after addition of 2% serum. 22/121 blood donors were persistent *S. aureus* carriers, 70 were noncarriers. Multiplex-PCR analysis revealed a highly heterogenous SAG gene repertoire of the colonizing strains. *S. aureus* carriers mount an efficient neutralising antibody response to SAGs, which is highly specific for their colonizing strain. In conclusion, *S. aureus* carriers show a very effective and highly specific neutralizing antibody response against the SAGs of their colonizing strain. This strain-specific immune response, which may be similar for other virulence factors, may explain why the outcome of *S. aureus* bacteremia is more favourable in carriers than in noncarriers.

Primarily interleukin 12-deficient primate dendritic cells migrate to lymphoid tissues and induce protein-specific T helper cell immune responses in vivo, possibly through secretion of IL-23

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Experimental studies in monkeys on the basis of in vitro generated, re-injected dendritic cells (DCs) allow investigating primate DC biology in vivo. To study the quality of immune responses induced by interleukin (IL)-12 deficient primate DCs in vivo, we adapted previous findings obtained in vitro with human cells to the rhesus macaque model. Following exposure of immature monocyte-derived monkey DCs to the immunomodulating synthetic polypeptide glatiramer acetate (GA) and, as a DC activating stimulus inhibiting the induction of T helper (Th) 1 immune responses, to the cyclic adenosine monophosphate (cAMP) enhancer, dibutyryl-cAMP (d-cAMP), the resulting DC population displayed a mature phenotype defined by characteristic surface molecule expression, reduced endocytosis, and enhanced antigen-specific T-cell stimulatory function, notably also for pre-primed Th1 cells. Phosphorylation of p38 mitogen activated kinase was not induced in GA/d-cAMP activated DCs. Accordingly, these cells secreted significantly less IL-12 ($p \leq 0.001$) than cytokine-activated cells. Two days after intradermal injection, GA/d-cAMP activated fluorescence-labeled DCs were detected in the T cell areas of draining lymph nodes. Similarly injected, GA/d-cAMP activated keyhole limpet hemocyanin (KLH)-loaded DCs induced Th immune responses characterized by KLH-specific IL-2 and IFN-gamma secretion by PBMCs. In addition, we detected IFN-gamma and TNF at single-cell levels in KLH-specific T cell lines, and IL-17 was detected in cell culture supernatants of these cell lines. Secretion of IL-23 upon CD40 ligation might contribute to the induction of antigen-specific Th immune responses by IL-12 deficient DCs in vivo.

Cytokine mRNA are expressed in distinct waves after immunization: Tracking immune responses in the T and B cell compartments of the spleen

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Because of its central position in the blood stream and the large amount of migrating lymphocytes, the spleen plays a central role in the primary defence against blood borne infections. In our study we monitored the course of an immune response in the spleen after i.v. injection of sheep red blood cells (SRBC). SRBC is a particulate antigen that gets trapped in the Marginal zone (MZ) very similar to the influx of capsulated bacteria. It is known that immune and nonimmune cells effectively communicate through the expression of cytokines. Using a combination of laser microdissection, Real Time RT-PCR and immunohistochemistry we are able measure the expression of cytokines within specific lymphoid compartments (T cell zone, B cell zone and MZ). The increased expression of *IL10* mRNA in the MZ and in the B cell zone indicates that the arriving antigen activates cells in both compartments. The increase of *IL12p40* mRNA 9h after immunization points to the activation of antigen presenting cells in the MZ and the T cell zone. In parallel the upregulation of *IL2* transcripts in the T cell zone marks the stimulation of antigen specific T cells. Finally, 24h after injection the increase in *IFN gamma* and *IL27p28* transcripts is restricted to the T cell zone. Our results clearly demonstrate that the transient and compartment-specific expression of cytokines can be used to chase the spatial and temporal sequence of events after immunization.

Differential proliferative response of PBMCs among tuberculosis patients to PPD and IFN-gamma

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One characteristic feature of human tuberculosis is the phenomenon of hypo-response to specific antigens, anergy. Low proliferation to specific antigens is correlated to decreased IFN-gamma, but increased IL-4 and IL-10 secretion in anergic tuberculosis patients. Regulatory T cells were recently described to play a substantial role in coordinating a balanced immune response in tuberculosis.

Therefore we aimed at studying different manifestations of the response of PBMCs from tuberculosis patients to specific antigens from *M. tuberculosis* PPD and to macrophage activator IFN-gamma.

Though the overall proliferation to PPD was elevated in patients, a high percentage of non-responders to PPD (21 patients out of 34) was detected. On this basis, we divided patients into two sub-groups: A – non-responders to PPD, and B – responders to PPD. There were no differences of PBMCs' oxidative burst between A and B tuberculosis patient sub-groups. However, patients from both subgroups showed diminished oxidative burst to PPD or IFN-gamma, when compared to healthy donors. Tuberculosis patients from group A also presented decreased capacity to secrete IL-2 and IFN-gamma in response to PPD, compared to patients from group B. Though there was no correlation of weak oxidative burst with diminished proliferation to PPD, proliferation to PPD correlated with IL-2 and IFN-gamma production in patients' PBMCs. We found that oxidative burst in response to PPD was positively correlated with IL-4 in the patient group but negatively correlated in the healthy donor group.

A Novel Mouse Model of *Bartonella*-Induced Cat Scratch Disease: Immune Mechanism Leading to Lymphadenopathy

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The Gram-negative bacterium *Bartonella (B.) henselae*, which has a feline reservoir host, is known as facultative intracellular pathogen causing cat scratch disease (CSD) in immunocompetent humans. The clinical manifestations of CSD are characterized by a long-lasting, but self-healing enlargement of the draining lymph node which rarely contains cultivable *Bartonella*. So far no data about the immunopathogenic mechanism leading to this lymphadenopathy exist. This prompted us to establish a mouse model of CSD. After subcutaneous infection with a high dose of *B. henselae* mice developed a striking and long-lasting swelling of the draining lymph node similar to CSD. It was more severe compared to that induced by other *Bartonella* species. The transfer of carboxyfluoresceinsuccinimidylester(CFSE)-labeled splenocytes and the administration of 5-Bromo-2'-deoxyuridine (BrdU) to *Bartonella*-infected mice revealed that a preferential influx of B-cells into the draining lymph node as well as cell proliferation contribute to the observed lymphadenopathy. In studies which addressed the function of different immune cells in vitro we could show that plasmacytoid dendritic cells exposed to *Bartonella* secreted high amounts of interferon (IFN)-alpha/beta. In vivo IFN-alpha/beta receptor deficient mice subcutaneously infected with a *Bartonella* species which in wildtype mice led to only mild alterations developed a severe local lymphadenopathy.

Thus, we conclude that altered immune cell recruitment and lymphoproliferation are involved in the pathogenesis of *B. henselae*-induced lymphadenopathy in mice. The only mild lymphadenopathy seen after infection with other *Bartonella* species may be due to an inhibitory effect of IFN-alpha/beta.

In vitro model of the blood-brain barrier to study the entry of *Toxoplasma gondii* into the brain

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Toxoplasma gondii is an intracellular protozoan that infects nucleated host cells by active penetration. Infection can be acquired by oral ingestion of cysts or oocysts from raw and undercooked meat or from contaminated soil. After rupture of cysts in the gut, the parasite disseminates all over the body and reaches the brain where - under the control of the immune system - the parasite persists for lifetime. In immunocompromized patients reactivation leads to lethal encephalitis if left untreated. The mechanisms of dissemination and entry of *Toxoplasma gondii* into the brain are unknown. We investigate the mechanisms of passage across the blood-brain-barrier and whether the parasite gains access to the brain as a free parasite or inside host leukocytes (“Trojan Horse”).

Using an in-vitro model of the blood-brain barrier consisting of primary rat brain endothelial cells co-cultured in a transwell chamber with primary rat brain astrocytes we characterize the transmigration of different subsets of naive and infected rat blood leukocytes. TEER and permeability are measured to control the integrity of the blood-brain barrier; functionality was assured by addition of MCP-1 to the lower compartment of the transwell system resulting in increased migration of monocytes across the filters. When uninfected PBMCs at a ratio of about 10% monocytes vs. 90% lymphocytes were added into the upper compartment of the transwell system for 3 hours, preferential migration (= recovery from the lower chamber) of the monocyte population (30% monocytes vs. 70% lymphocytes) was observed. When PBMCs were infected in vitro for 1 hour with *Toxoplasma gondii*, the percentage of infected monocytes was 5 to 10 times higher than the percentage of infected lymphocytes, resulting in higher absolute numbers of infected monocytes than lymphocytes. Preliminary results also indicate that 3 hours after the addition of in-vitro infected PBMCs to the upper compartment of the transwell system not only the ratio of monocytes vs. lymphocytes in the lower compartment had changed but also the percentage of infected vs. uninfected monocytes had increased.

In conclusion, monocytes are preferentially infected by *T. gondii* in peripheral blood and migrate through the blood-brain barrier in vitro. Our approaches will enhance the knowledge of the interaction of the parasite and host cells with the blood-brain barrier.

Depletion of regulatory T cells in DEREK mice leads to Ovalbumin-specific immunity without adjuvants

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Stimulation of Toll-like receptors (TLRs) leads to activation of antigen presenting cells (APCs), resulting in the induction of protective immunity. Activation of dendritic cells (DCs) is the basis of potent adjuvants like CpG DNA, whereas immunization with ultra-purified proteinaceous antigens fails to induce immunity. Microbial induction of TLR signalling has been demonstrated to overcome the suppressive effect of natural Tregs, allowing activation of pathogen-specific adaptive immune responses. The reverse approach, to deplete Tregs during an ongoing immune response to induce immunity in the absence of adjuvants has been hampered by technical limitations since common protocols using anti-CD25 antibodies also depleted activated conventional T cells. Here, we describe a novel bacterial artificial chromosome (BAC) transgenic mouse model (DEREG mice: DEpletion of REGulatory T cells) expressing a diphtheria toxin receptor eGFP fusion protein under the control of the *foxp3* locus for selective depletion of Foxp3^+ Tregs by diphtheria toxin (DT) injection. Ablation of Tregs in adult mice after subcutaneous vaccination with LPS-free ovalbumin (OVA) led to clonal expansion of Ag-specific IFN γ -producing CD8 T cells. We are currently testing if depletion of Tregs leads to OVA-specific T cell memory without DC activation. Further, DEREK mice allow us to investigate the role of Tregs in the priming phase of the immune system towards various infections. Dissecting the role of Tregs during different phases of the immune response will shed light on the functional interplay between Tregs and DCs and may have important consequences for the development of vaccines against tumour antigens and infectious diseases.

Granulocyte macrophage – colony stimulating factor expression characterizes a novel *Mycobacterium tuberculosis* specific CD4⁺ memory T cell population

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Characterization of the T cell response essential for protective immunity against *Mycobacterium tuberculosis* and long-lasting memory has major implications for vaccine development and immune intervention based strategies. The basic assumption underlying the present study is that different cytokine pattern expressed by distinct memory T cell subpopulations might serve as reliable biomarkers for natural or vaccine induced protection. In addition, such markers could improve intricate diagnosis in childhood tuberculosis (TB). We determined the expression of IFN-gamma, IL2, TNF-alpha, and GM-CSF in T cell subpopulation from children with TB and healthy latently *M. tuberculosis* infected contacts (LTBI) after *in vitro* stimulation with *M. tuberculosis* protein extract and specific proteins. We identified CD4⁺ effector memory T cells (T_{EM}) (CD45RO^{high}, CD62L^{low}) as the major source of all measured cytokines in both study groups. Higher proportions of IFN-gamma, TNF-alpha and IL2 expressing cells in children with TB were observed for T_{EM} while only minor differences were detected for T_{CM} after stimulation with *M. tuberculosis* protein extract. GM-CSF secretion was more strongly dependent on antigen-specific stimulation as compared to other cytokines. Analyses of multi cytokine pattern revealed that the majority of GM-CSF positive T_{EM} were also expressing IFN-gamma and TNF-alpha similar between study groups but a tendency of increased subpopulation proportion of GM-CSF expressing triple positive T_{EM} in LTBI was detected. We conclude that children with acute TB had higher proportions IFN-gamma, TNF-alpha and IL2 positive T_{EM} while GM-CSF co-expression identified a novel subpopulation within CD4⁺ T_{EM} with possible impact on protective immunity against *M. tuberculosis*.

Differential dendritic cell modulation by commensal bacteria *in vivo*

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As IL-2-deficient (IL-2^{-/-}) mice mono-colonized with *E. coli* develop colitis but IL-2^{-/-} mice mono-colonized with *B. vulgatus* do not, we investigated if mono-colonization with *E. coli* or *B. vulgatus* differentially modulates distribution, activation and maturation of intestinal lamina propria (LP) dendritic cells (DC). Prior to onset of colitis we found increased expression of the activation marker CD40 on LP DC from *E. coli* but not *B. vulgatus* mono-colonized mice but high MHC class II expression by DC from both groups. Chemokine receptor (CCR) 7 surface expression was more strikingly enhanced in mesenteric lymph node (MLN) DC from *E. coli* than *B. vulgatus* mono-colonized mice. High IL-6 mRNA expression was evident in *B. vulgatus* mono-colonized IL-2^{-/-} mice that did not develop colitis. *B. vulgatus*-mediated suppression of CCR7 expression and DC migration depends on IL-6 as shown by experiments with IL-6^{-/-} DC. *B. vulgatus*-triggered IL-6 secretion by LP DC thus induces a semi-mature phenotype of DC characterized by low expression of CD40 and CCR7 but high expression of MHC class II that prevents colitogenic T cell activation. The data provide new evidence that IL-6 can act as an anti-inflammatory cytokine in the mucosa by targeting local DC.

IL-13 complements the pathogenic effect of IL-4 upon pulmonary infection of mice with *Cryptococcus neoformans*

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In the murine model of *Cryptococcus neoformans* infection Th1 (IL-12/IFN-gamma) and Th17 responses (IL-23/IL-17) are associated with protection from infection with the opportunistic pathogen, whereas an IL-4-dependent Th2 response is the cause of disease progression. In order to investigate the role of the Th2-cytokine IL-13 and compare its activity with that of IL-4 during pulmonary infection with a highly virulent *C. neoformans* strain, intranasally infected IL-13^{-/-}, IL-13-transgenic (IL-13^{T/+}) and wild-type (WT) mice were studied. For comparison IL-4^{-/-} and IL-4R^{-/-} (nonresponsive to IL-4 and IL-13) mice were also examined. Infected IL-13^{T/+} mice had a reduced survival time as compared to WT mice. In contrast, infected IL-13^{-/-}, IL-4^{-/-} and IL-4R^{-/-} were highly resistant and survived during the entire period of the experiment. Organ burdens of lung, brain and spleen were considerably higher in IL-13^{T/+} mice than in WT mice. We found functional pulmonary impairment in the presence of IL-13 as evidenced by enhanced airway hyperreactivity in IL-13^{T/+} and WT mice in comparison to resistant genotypes. Secretion of IL-13 upon infection with *C. neoformans* was associated with hyperplasia and elevated mucus production by goblet cells. When IL-13 is overexpressed, a strong and diffuse inflammatory response develops in lung and brain. High levels of serum IgE and low ratios of serum IgG2a/IgG1 were found in susceptible WT and IL-13^{T/+} mice. This indicates that IL-13 contributes to Th2-induced pathogenesis. To analyze whether IL-13 induces formation of alternatively activated macrophages we currently determine expression of macrophage mannose receptor vs. expression of iNOS by classically activated macrophages.

Analysis of eosinophil development, recruitment and turnover *in vivo*.

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Eosinophils, effector cells of the innate immune system, are associated with Th2-mediated immune responses like allergic inflammation or parasite infections. We characterized development, recruitment and turnover of eosinophils under steady state conditions or during infection with the helminth parasite *Nippostrongylus brasiliensis* by flow cytometry using different surface markers like FIRE, CCR3 and Siglec-F. The inhibitory receptor Siglec-F was upregulated on peritoneal and lung eosinophils after infection with *N. brasiliensis*. Interestingly, SiglecF^{lo} but not Siglec-F^{hi} eosinophils in the lung showed rapid BrdU incorporation indicating that the latter cells were recruited from other organs without further cell division. To analyze homing and survival, eosinophils were adoptively transferred to naïve or infected recipient mice. Transferred eosinophils rapidly disappeared from blood and spleen and accumulated in the peritoneum, where they survived for several days. Pre-treatment with pertussis toxin blocked the recruitment to the peritoneum but not to other organs indicating that recruitment to the peritoneum is critically dependent on a G-protein coupled receptor. Small numbers of eosinophils could also leave the peritoneum and recirculate to other organs. Peritoneal eosinophils showed higher spontaneous eosinophil peroxidase release as compared to splenic eosinophils indicating a higher activation status of eosinophils in the peritoneum. Finally, immunofluorescence staining for Siglec-F allowed us to identify eosinophils by histology in the spleen where they accumulated in the red pulp in close association to the marginal zone.

***Mycobacterium tuberculosis*-induced cell death of primary human monocytes and macrophages is not significantly modulated by TNF-targeted biologicals**

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Differential induction of cell death in mycobacteria-infected monocytes and macrophages has been invoked as one possible mechanism by which some TNF-targeted biologicals reactivate tuberculosis more frequently than others. We infected primary human monocytes and monocyte-derived macrophages with the virulent *Mycobacterium (M.) tuberculosis* strain H37Rv and followed the rate of cell death in the absence or presence of a wide concentration range of four different TNF-targeted biologicals: infliximab and adalimumab (both monoclonal antibodies to human TNF) and etanercept and PEGsTNFR1 (both fusion constructs of human TNFR2 and TNFR1, respectively). None of the TNF-targeted biologicals used modulated the death rate of monocytes/macrophages induced by infection with *M.tuberculosis* alone. Our data support the view that mycobacteria-induced cell death is largely independent of TNF and that the primary target of differential modulation by TNF-targeted biologicals during tuberculosis is not a recently recruited monocyte or freshly differentiated macrophage.

Vaccination with plasmacytoid dendritic cells induces protection against *Leishmania major* in mice

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Vaccination with myeloid dendritic cells (DC) against *Leishmania major* induces a parasite-specific Th1 response and long-lasting protective immunity in susceptible mice. Since distinct DC subsets have been proposed to direct the predominant development of either Th1- or Th2-type T cell responses, we analyzed the capability of plasmacytoid DC (pDC) to induce protection and elicit a Th1 response against *L. major*. Pulsing with *L. major* lysate induced the activation and maturation of semi-mature murine pDC that had been isolated from the spleen, as indicated by up-regulation of the co-stimulatory molecules CD86 and CD80, but did not enhance the level of interferon-alpha secretion by pDC. Vaccination of susceptible mice with *L. major* lysate-pulsed pDC induced highly effective immunity against a subsequent challenge infection with *L. major* promastigotes. Surprisingly, the protection was not accompanied by a polarized Th1 cytokine profile, although protected mice showed a lower ratio of *Leishmania*-specific IgG1 to IgG2a than mock-treated control groups. Co-activation of pDC with CpG-containing oligodeoxynucleotides, which has been shown to be critical for activating the protective potential of myeloid DC, was not required for the protective effect of *L. major* antigen-pulsed pDC. These findings demonstrate that antigen-loaded pDC are able to mediate protection against a parasite disease and that experimental leishmaniasis is a suitable model to elucidate the mechanisms underlying DC-based vaccination against infections.

Immunostimulatory properties of highly purified peptidoglycan

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Several studies on pattern recognition of peptidoglycan (PGN) indicate signalling via TLR2. This was shown using TLR2-transfected HEK293 cells and then confirmed by using TLR2 knock-out mice. A study by Travassos et al. (EMBO 2004) questioned these results, showing that highly purified PGN from cell walls of several Gram-positive and Gram-negative bacteria does not signal via TLR2. To study the relative contribution of PGN-associated components (lipoteichoic acid (LTA), teichoic acid (TA), and lipoprotein) to immune activation by PGN, we established the preparation of highly purified PGN: A method based on boiling with SDS, removing contaminating proteins, LTA, and lipopeptides. Afterwards, treatment with small glass beads (>100 nm) separates cellular debris, and treatment with different enzymes removes DNA and RNA, cleaving sugars, peptides and ester bonds. The final step removes secondary polysaccharides covalently bound to PGN via phosphodiester bonds by treatment with hydrofluoric acid (HF). Concurring with the results published by Travassos et al., the thus purified PGN retained only very low cytokine-inducing activity in incubations of blood leukocytes, which was not TLR2- or TLR4-dependent. Subsequently, this method shall be used to prepare PGN from bacterial mutant strains, lacking either LTA (*S. aureus 113 delta-dlt*), lipoprotein (*S. aureus 113 delta-lgt*), or TA (*Listeria monocytogenes, delta-TA*): Preliminary data indicate that PGN preparations from *S. aureus delta-dlt* are less immunostimulatory at the start of the preparation when compared to its wild-type, whereas the purified PGN at the end of the preparation demonstrates no difference to the wild-type in its immunostimulatory properties.

Protein kinase C-theta critically regulates the induction of *Listeria monocytogenes*-specific CD4 and CD8 T cells

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T cells play an important role in the control and elimination of *Listeria monocytogenes* (LM) in murine listeriosis. Protein kinase C-theta is a Ca²⁺-independent member of the PKC family specifically expressed in T cells that plays an essential role in activation of mature T cells via stimulation of AP-1 and NF-kappaB. In viral infectious disease, PKC-theta-deficiency of T cells is compensated by activated dendritic cells and proinflammatory cytokines resulting in a normal T cell response and viral clearance.

To study the role of PKC-theta in murine listeriosis, C57BL/6 PKC-theta^{-/-} and wild type (WT) mice were i.p. infected with LM. Frequencies of LM-specific CD8 T cells specific for MHC class Ia and Ib-restricted epitopes as well as LM-specific CD4 T cells were drastically reduced in PKC-theta^{-/-} mice compared to WT animals. Upon reinfection secondary expansion of these LM-specific T cells remained reduced in PKC-theta^{-/-} mice. Consequently, the bacterial burden was increased in the spleen and the liver after primary and secondary infection with LM. In contrast to infection with viable LM, an immunization with mature WT CD11c⁺ bone marrow-derived cells loaded with LLO₁₉₀₋₂₀₁ and ova₂₅₇₋₂₆₄ peptides did only induce LM-specific T cells in WT, but not in PKC-theta^{-/-} mice. Adoptive transfer experiments with PKC-theta^{-/-} transgenic T cells revealed that PKC-theta is important for Ag-induced T cell proliferation and activation in listeriosis. In conclusion, this study demonstrates that during LM infection PKC-theta is critical for induction and development of pathogen-specific CD4 and CD8 T cells in vivo.

Regulatory processes in lyme borreliosis: effect of Transforming Growth Factor-beta

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Borrelia burgdorferi, a bacterium of the order *Spirochaetales*, is the causative agent of Lyme borreliosis, a frequently diagnosed infection in humans and animals. After transmission by ticks, the bacteria cause acute as well as chronic inflammatory reactions, especially in joints, in the pericardium and meninges.

TGF-beta (Transforming Growth Factor-beta), an immune modulating cytokine, plays a central role in controlling the activity of different cells of the immune system. Thereby, the pleiotropic factor elicits both immune suppressive as well as stimulating effects.

The project aims to determine the TGF-beta-expression in the course of Lyme borreliosis infection in the mouse. Furthermore, the influence of TGF-beta susceptibility and TGF-beta-production of T-cells on the borreliosis infection is being analysed by means of transgenic mouse models.

The investigations should lead to a better understanding of the mechanistic development of chronic inflammation caused by borrelia infection. This is the precondition for obtaining diagnostic tools which would allow for a prediction of the progressivity of chronic inflammatory reactions – as well as for developing adequate therapeutical agents.

New vaccination strategies against infectious diseases: Microbial antigen expression by RNA-electroporated dendritic cells

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Dendritic cells have the unique ability to stimulate resting T cells and initiate a specific cellular immune response. These potent immunostimulatory properties make them attractive candidates for novel vaccination and immune intervention strategies. Using the model of murine leishmaniasis, we were able to demonstrate that bone marrow-derived dendritic cells (BMDC), after activation with CpG-containing oligonucleotides and pulsing with *Leishmania major* antigen, induce a parasite-specific Th1 immune response that protects otherwise susceptible mice against the disease. In order to further enhance this approach, we started to investigate the potential of BMDC to induce an anti-leishmanial immune response after electroporation with *in vitro* transcribed *L. major* antigen-encoding mRNA. Proliferation assays with antigen-specific T cells suggest that fusion of the antigens with the lysosome-targeting signal of the lysosome-associated membrane protein-1 (LAMP-1) results in substantially increased presentation of peptides by MHC class II molecules. Furthermore, we investigated whether costimulation of the electroporated BMDC by CpG-containing oligonucleotides, lipopolysaccharide or CD40L increased their immunostimulatory capacity. Although T cell proliferation was not increased further, preliminary results indicate that CD40L is able to enhance the secretion of Th1-mediating cytokines. Taken together, these results indicate that mRNA-transfected BMDC can effectively be manipulated to induce T cell proliferation which is a prerequisite for their use as effective vaccine carriers to induce immunity against a protozoan parasite.

Murine APC in *Salmonella* infection: Task sharing between DC and macrophages

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IL-12 is essential for protection against *Salmonella* infection. Several studies suggested macrophages (Mph) to be producers of IL-12 in response to *Salmonella*. However, more recent data point to dendritic cells (DC) as important target cells for *Salmonella* and as a source for IL-12. In this study DC and Mph are compared in terms of the release of the IL-12 family members IL-12, IL-23, p40 and the bactericidal effector molecule NO in response to *Salmonella* Enteritidis (SE) *in vivo* at the site of infection and the draining lymph node (DLN).

We infected mice intraperitoneally with SE. After 1 and 14 days the release of IL-12, IL-23 and p40 at the site of infection (i.e. in peritoneal lavage fluid) and in culture supernatants of *ex vivo* stimulated peritoneal exudate cells (PEC) was analysed. In addition, to study cytokine production at the site of immune response induction, cells from the lymph node draining the peritoneal cavity were analyzed for production of IL-12 family members. To identify the cell type responsible for production of IL-12 family members and of NO at the site of infection we depleted either DC or Mph from PEC and analysed the *ex vivo* release of p40, IL-12 and NO.

We show that in response to SE IL-12, p40 and NO but not IL-23 are primarily released at the site of infection. The main producers of p40 in response to SE at the site of infection are DC, whereas Mph are more potent producers of NO.

Interaction of lipoteichoic acids with components of human plasma

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Lipoteichoic acid (LTA) is a major immunostimulatory component of Gram-positive bacteria. In contrast to LPS, the main immunostimulatory principle of Gram-negative bacteria, little is known about binding-proteins for LTA in human plasma. The aim of this study was to identify such interaction partners and to characterize their effects on the cytokine inducing capacity of LTA. We analyzed SDS-PAGE profiles of proteins from human serum that was fractionated by gel chromatography in the presence or absence of LTA. Band shifts indicated interaction of some serum proteins with LTA. Two of these proteins were identified by MALDI-TOF mass spectrometry as the apolipoproteins A2 and B100. Binding assays were performed to confirm the interaction. Microtiter plates were coated with Apo-A2 or Apo-B100 and binding of biotin labelled LTA was quantified. To study the effect of apolipoproteins on cytokine induction we stimulated human serumfree PBMCs with LTA in the presence or absence of purified apolipoproteins. Apo-B100 inhibited LTA-induced TNF and interleukin-1-beta release while cytokine induction was not influenced by Apolipoprotein-A1. These experiments suggest that the ability of apolipoproteins to neutralize LTA may play a role in the immune defence against Gram-positive bacteria.

Auswirkungen der DC-SIGN Ligation auf Vakzinierungen

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Dendritische Zellen (DZ) besitzen durch ihre zentrale Funktion als wichtigste professionelle Antigen-präsentierende Zellen eine Schlüsselrolle innerhalb des Immunsystems. Abhängig von Rezeptor-vermittelter Antigenaufnahme und DZ-Aktivierung über Mustererkennungsmoleküle (zB. TLRs) kann sowohl Immunität als auch Toleranz induziert werden.

Wie am Beispiel von DEC205 gezeigt wurde, kann durch Stimulation oder Blockierung spezifischer Antigenrezeptoren aus der Familie der C-Typ Lektine, die Immunantwort gezielt in Richtung Immunität oder Toleranz dirigiert werden.

In diesem Zusammenhang untersuchen wir die Funktion des humanen Lektin-Rezeptors DC-SIGN (CD209), der mit Glykoproteinen auf der Oberfläche zahlreicher Mikroorganismen wie beispielsweise *Mycobacterium tuberculosis*, *Helicobacter pylori* und HI Virus interagiert. Zu diesen Glykoproteinen zählt unter anderem das Lewis-Antigen. Durch Konjugation von Lewis-Antigen mit dem Modellantigen Ovalbumin (Ova) können wir Vakzinierungsexperimente im Mausmodell durchführen, die spezifisch auf DC-SIGN abzielen. Wir wollen untersuchen, welche Form der Immunantwort gegen das Modellantigen Ovalbumin nach Aufnahme über humanes DC-SIGN induziert wird. Zur Analyse der Rolle von humanem DC-SIGN *in vivo* haben wir konventionelle transgene Mäuse generiert („hSIGN“), die diesen Rezeptor analog zum Menschen auf CD11c⁺ DZ exprimieren.

Erste Ergebnisse deuten darauf hin, dass die Vakzinierung mit dem Lewis-Antigen/Ovalbumin Konjugat bei transgenen hSIGN Mäusen im Vergleich mit Wildtyp Mäusen zu einer gesteigerten Expansion von Ovalbumin spezifischen CD8⁺ T-Lymphozyten führt. In weiteren Vakzinierungen werden wir CD4⁺ T-Lymphozyten und Ovalbumin-spezifische Antikörper-Profile untersuchen.

Deviation of dendritic cell development by Toll-like receptor stimulation is associated with the expression of suppressors of cytokine signalling (SOCS)

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Dendritic cells (DCs) are known to be the sentinels of the immune system: Part of innate immunity they are sensing the presence of infectious microorganisms. Upon detection of pathogens, DCs undergo several functional changes and finally activate adaptive immunity. These processes are reflected by the fact that stimulation of Toll-like receptors (TLRs) (e.g. with lipopolysaccharide) transforms immature to mature DCs. However, TLRs are also expressed on precursor cells of DCs.

Here we analyzed the effects of TLR stimulation during the process of granulocyte-macrophage-colony-stimulating factor (GM-CSF)-mediated *in vitro* generation of immature DCs from precursor cells. We show that TLR triggering deviated phenotypic (surface molecule expression) and functional (phagocytotic and T cell stimulation capacity) differentiation from CD14⁺ monocytes to CD1a⁺ DCs. Comparable results were obtained when differentiation of murine myeloid DCs from bone marrow cells was analyzed.

The observed effects were TLR-dependent but independent of soluble factors. TLR stimulation in DC precursor cells induced proteins of the suppressor of cytokine signalling family (SOCS), correlating with the loss of sensitivity to GM-CSF. Additionally, GM-CSF signal transduction was abolished by overexpression of SOCS-1. Furthermore, forced SOCS-1 expression in DC precursors mimicked the inhibitory effects on DC generation observed for TLR stimulation.

Taken together our data indicate that TLR stimulation during the period of DC generation interferes with and deviates DC differentiation and that these effects are mediated by SOCS.

Do *Leishmania spp.* use apoptotic mimicry or altruistic behaviour for the establishment of a productive infection?

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An early marker for apoptosis is the exposure of phosphatidylserine (PS) on the outer leaflet of the cell membrane. Uptake of PS-positive apoptotic cells by professional phagocytes results in a silencing of the immune response. For *Leishmania spp.* PS-exposure is an essential survival factor. Two evasion strategies involving PS-exposure have been demonstrated. For amastigotes from *L. amazonensis* a strategy termed “apoptotic mimicry” shows that all amastigotes express PS without subsequent apoptosis. In contrast, for promastigotes from *L. major* an apoptotic PS-positive subpopulation enables survival of a PS-negative population in an altruistic manner. In this study we investigated PS-expression and apoptosis of *Leishmania* amastigotes. We compared promastigotes and amastigotes derived from *L. major* and *L. donovani*.

3 days after *L. major* infection of human macrophages we found the intracellular presence of both TUNEL-positive and -negative parasites. In addition, we found that purified *L. major* amastigotes, taken from footpads of 8 week infected BALB/c, contained both TUNEL-positive and -negative parasites populations. Subsequently we analysed PS-expression and TUNEL-positivity on both *L. donovani* promastigotes and axenic amastigote cultures. We found that *L. donovani* promastigote cultures become PS-positive like *L. major* promastigotes. Moreover we could demonstrate that *L. donovani* axenic amastigote cultures contain a PS-positive and TUNEL-positive subpopulation. In contrast to *L. amazonensis* amastigotes, with *L. major* and *L. donovani* we find two populations of apoptotic and non-apoptotic amastigotes. Our data suggest that next to apoptotic mimicry amastigotes can also use altruism for the establishment of a productive infection.

***Chlamydia pneumoniae* uses apoptotic polymorphonuclear neutrophil granulocytes to enter into macrophages**

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Phagocytes like polymorphonuclear neutrophil granulocytes (PMN) and macrophages (MF) kill pathogens as the first line of defense. Pathogens have developed strategies to silently infect their preferred host phagocytes. The best example of an immune silencing phagocytosis process is the uptake of apoptotic cells. Immune responses are suppressed by the recognition of phosphatidyl serine (PS) on the membrane of apoptotic cells.

We found that *Chlamydia pneumoniae* (*Cp*) can infect and survive inside PMN. Infection with *Cp* delayed the spontaneous apoptosis of PMN but after 66 h even the infected PMN became apoptotic and PS-positive. Importantly, these apoptotic PMN still harbour viable and infective bacteria. In addition we observed that infected PMN secreted high levels of the monocyte-attracting chemokine MIP-1 β . Since a major function of MF is the clearance of apoptotic cells, we investigated whether MF can ingest apoptotic PMN that harbour intracellular *Cp*. We observed that *Cp* internalized by this indirect way survived and multiplied inside MF and from these cells the infection could be transferred to epithelial cells. In contrast, direct ingestion of *Cp* by MF resulted in a persistentlike state of infection. In addition to the indirect transfer we observed that apoptotic PMN released PS-positive “bleb like” structures containing *Cp*. Our data suggest that *Cp* uses PS-positive PMN and possibly PS positive blebs to transfer to other host phagocytes without being killed.