3rd International Symposium on ”Autoinflammation breaks Barriers”

Report of Contributions

Contribution ID: **10** Type: **Poster**

Relevance of neutrophil extracellular traps during vessel damage in skin-limited IgA immune complex vasculitis

Immune complex vasculitis is a vascular inflammation that mainly affects small blood vessels. Initial steps are formation of immune complexes in the vessel, followed by neutrophil and IC interaction, accumulation, deposition, activation and ensuing destruction of vessel wall. It is unknown how and which cytotoxic components cause the vessel damage. Stimulated neutrophils produce extracellular structures called neutrophil extracellular traps. NETs are filaments of decondensed chromatin associated with proteins like histones, elastase and myeloperoxidases which can have a cytotoxic effect to the endothelium. NETosis is stimulated by several molecules, like ICs, but the relevance in importance in skin-limited IgA ICV is unknown. The objective was to investigate the impact of NETs from human neutrophils on vessel damage during skin-limited IgA ICV in vivo, in vitro and ex vivo.

We were able to show together with deposited neutrophils a presence of cytotoxic NET structures (H3Cit, MPO and Ela) in lesion skin. We confirmed that neutrophils of skin-limited IgA ICV patients are able to release high amounts of NET after stimulation with IgA ICs or PMA in comparison to healthy donor neutrophils. That's why we assumed, that neutrophils of skin-limited IgA ICV patients are primed and examined serum samples from the patients. We found and elevated level of IL-6, IL-18 and S100A8/S100A9 and were able to show an enhanced reaction potential of healthy donor neutrophils to release NET after pre stimulating the cells with IL-6 or S100A8/S100A9. IL-18 showed no effect on the NETosis rate. In vitro we were able to evaluate the highly cytotoxic effect of NET released from skin-limited IgA ICV patients. To reconstruct the in vivo conditions we used a shear-flow system and showed an aggregation of neutrophils after addition of IgA ICs followed by deposition of these aggregates to the activated endothelium under low-flow. Accompanying the released NET was highly cytotoxic to the endothelium under flow if the DNA structure was not destroyed by DNase. In our mouse model a degradation of DNA after inducing vasculitis, by passive reverse Arthus reaction, resulted in an improved disease outcome. These findings indicate the importance of neutrophils and NET during the evolvement of vessel damage in skin-limited IgA ICV.

Primary author: Mrs MAYER-HAIN, Sarah (Instiut für Immunologie)

Co-authors: Mr NEUFELD, Matthias (Klinik für Hautkrankheiten - Allgemeine Dermatologie und Venerologie); Dr EHRCHEN, Jan M. (Klinik für Hautkrankheiten - Allgemeine Dermatologie und Venerologie); Prof. VOGL, Thomas (Institut für Immunologie); Dr PAPPELBAUM, Karin I.; Prof. SUNDERKÖTTER, Cord (Universitätsklinik und Poliklinik für Dermatologie und Venerologie)

Presenter: Mrs MAYER-HAIN, Sarah (Instiut für Immunologie)

Contribution ID: **11** Type: **Talk**

The alarmin S100A8/S100A9 is a key regulator of monocyte activation and homing

Phagocytes are fast migrating cells throw-out the body and are important for the pathogen defense. Therefore these cells have to migrate and transmigrate to sites of infection in a coordinated way. The cellular dynamics underlying inflammatory activation of monocytes are regulated by signaling pathways that involve changes in intracellular calcium, GTPase activation and specific protein kinases. The aim of this project is to unravel the function of the major calcium binding protein complex S100A8/S100A9, using Lifeact Hoxb8 monocytes from wild-type and S100A9 knock-out mice. S100A8/S100A9 heterodimers are well studied alarmins involved in inflammatory processes by triggering TLR-4 dependent pathways. Moreover, these proteins are known to induce neutrophil adhesion by activation of β2 integrins.
However, mechanisms to keep these cells in the resting state are far less understood.
Interestingly, we found major alterations in the cytoskeletal dynamics and morphology of S100A9 knock-out cells. Already in the resting state, S100A9 knock-out monocytes showed faster migration and transmigration rates, reduced adhesion properties, lower traction forces and an elevated activation level of Rho GTPases compared to wild-type monocytes. Surprisingly after stimulation with chemokines, only the wild-type cells were able to respond and increase their migratory activity whereas the S100A9 knock-out cells seem to be already pre-activated and remained unaffected by chemokine stimulation. Substitution of missing extracellular S100A8/A9 in S100A9 knock-out cells reversed the phenotype again to a resting state. Our findings suggest that the extracellular S100A8/S100A9 control monocyte dynamics through the binding to the novel S100 receptor CD69 on monocytes.

Primary author: Mrs RUSSO, Antonella (Institute of Immunology)

Co-authors: Mrs FISCHER-RIEPE, Lena (Institute of Immunology); Mr SCHÜRMANN, Hendrik (Institute of Cell Biology); Mr BRANDT, Matthias (Institute of Cell Biology); Prof. BETZ, Timo (Institute of Cell Biology); Prof. ROTH, Johannes (Institute of Immunology); Prof. VOGL, Thomas (Institute of Immunology)

Presenter: Mrs RUSSO, Antonella (Institute of Immunology)

Contribution ID: **12** Type: **Poster**

Cutaneous over-expression of RANKL leads to suppression of anti-microbial immune responses

Cutaneous infections are controlled by the skin immune system eliciting innate as well as adaptive anti-microbial immune responses. Different studies, including our work, revealed that critical involvement of the tumor necrosis factor (TNF)/TNF receptor superfamily are regulating the cutaneous immunity. However, to complete their life cycle, pathogenic microorganisms need to suppress host anti-microbial immune responses. In particular CD4+Foxp3+ regulatory T cells (Treg) and MDSCs play a crucial role in the inhibition of cellular immunity. The molecular mechanisms determining the activation, expansion or migratory behaviour of Treg and MDSCs during cutaneous infections are still poorly understood. Upon intradermal infection with S. aureus in the back skin, K14-RANKL tg mice exhibited an increased skin lesion size as well as bacterial load compared to WT controls. This effect was mediated by increased abundance of Treg in K14-RANKL tg mice. To address the in-vivo relevance of Treg for the suppression of anti-bacterial immunity in S. aureus, K14-RANKL tg mice were bred with DEREG mutants, expressing the diphtheria toxin receptor (DTR) under control of the Foxp3 promoter. Subsequently, Treg were depleted in K14-RANKL tg x DEREG double mutants by intraperitoneal injection of diphtheria toxin (DT) before and after S. aureus infection. This results in the accumulation of G-MDSCs (granulocytic Ly6Ghigh Ly6Clow) and downregulation of M-MDSC (monocytic Ly6C+). Together, this data indicates that RANK-RANKL signaling increases Treg frequencies that lead to suppression of host anti-microbial immune responses while Treg deficiency results in an increase of G-MDSCs and the suppression of M-MDSC populations.

Primary authors: Dr MOGANTI, Kondaiah (1Department of Dermatology University of Münster, D-48149 Münster, Germany); Dr KLENNER, Lars (Department of Dermatology University of Münster, D-48149 Münster, Germany); Prof. LUGER, Thomas A (1Department of Dermatology University of Münster, D-48149 Münster, Germany); Prof. STEINBRINK, Kerstin (Department of Dermatology University of Münster, D-48149 Münster, Germany); Prof. LOSER, Karin (Department of Dermatology University of Münster, D-48149 Münster, Germany)

Presenter: Dr MOGANTI, Kondaiah (1Department of Dermatology University of Münster, D-48149 Münster, Germany)

Contribution ID: **13** Type: **Poster**

The Yersinia outer protein M (YopM) reduces keratinocyte hyperproliferation and neutrophil infiltration psoriasis-like inflammation in mice

Psoriasis is an inflammatory skin disease affecting around 2-4 % of the western European population. Patients suffer from red, itchy and scaly plaques and an impaired quality of life. Several treatment options are available, but with higher efficacy often toxicity and/or therapy costs are rising.

The Yersinia outer protein M (YopM) was the first bacterial effector protein discovered to be a cell-penetrating peptide (CPP), meaning it is able to autonomously enter eukaryotic cells independent of the T3 secretion system. It has been demonstrated that recombinant YopM (rYopM) down-regulated the expression of pro-inflammatory cytokines, like TNF-alpha or IL-1beta, after penetration of the host cell. Current theories about the mechanism of action focus on the inhibition of caspase-1 activation by either binding directly to pro-caspase-1 or to the scaffolding protein IQGAP1 and the downstream inhibition of the formation of the NLRP3 inflammasome. Hence, rYopM might be a promising molecule for further investigation in the context of local therapies for inflammatory skin diseases like psoriasis.

Subcutaneous injection of full-length rYopM already showed promising results in ongoing Imiquimod (IMQ)-induced psoriasis-like skin inflammation in mice. To narrow down the functional groups of the protein a truncated version of the protein was generated where only the two N-terminal α-helices (essential for translocation) and the first three leucine-rich repeats (LRRs) are present, called rYopMLRR1-3. As a negative control a construct wherein only the first three LRRs are missing was used, termed rYopMΔNLS1. To elicit psoriasis-like inflammation in mice, the animals were creamed daily with 62.5 mg of Aldara, containing 5 % IMQ on the shaved lower back for 8 consecutive days. Following disease onset the mice were additionally injected with 50 µg rYopM, rYopMLRR1-3, rYopM ΔNLS1 or PBS as control on a daily basis. Throughout the experiment the clinical score of the mice was determined and on the final day of the experiment skin samples were taken for histological examination, RNA analysis and protein quantification. The results clearly showed a reduced clinical score and scratching behavior of the rYopM and rYopMLRR1-3 treated animals, which was reflected in the reduced epidermal thickness as assessed by hematoxylin & eosin staining. Furthermore, keratinocyte proliferation was examined by the means of Ki-67 stainings and was markedly decreased in these groups resulting in lower levels of acanthosis. The quantification of inflammatory protein levels in the skin revealed reduced concentrations of pro-inflammatory and psoriasis-associated cytokines, like IL-17A, TNF-alpha or IL-1beta, as well as neutrophil-attracting chemokines, like CXCL1 and CXCL5. This finding was also confirmed by RNAseq analysis, suggesting a similar mode of action of rYopM and rYopMLRR1-3. Consequently, rYopM and rYopMLRR1-3 treatment significantly impaired neutrophil immigration into affected skin areas. Therefore, the anti-inflammatory capacity of rYopM treatment in psoriasis-like skin inflammation has clearly been shown. In addition, the essential part of rYopM for mediating this effect was narrowed down to the first three leucine-rich-repeats.

To sum this up, YopM is a promising target for further analysis in the context of local treatment of inflammatory skin diseases.

Primary author: Mrs SCHNEEWEISS, Maria (Department of Dermatology)

Co-authors: Prof. LOSER, Karin (Department of Dermatology); Prof. SCHMIDT, Alexander (Center for Molecular Biology of Inflammation); Dr RÜTER, Christian (Center for Molecular Biology of Inflammation); Dr POCEVA, Marija (Center for Molecular Biology of Inflammation); Dr GOSSENS, Anabel (Center for Molecular Biology of Inflammation)

Presenter: Mrs SCHNEEWEISS, Maria (Department of Dermatology)

Contribution ID: **14** Type: **Poster**

NLRP3 inflammasome activity in monocytes is regulated by 12/15-lipoxygenase.

Background: Activation of the NLRP3 inflammasome is a major inflammatory pathway in monocytes in response to various exogenous and endogenous stimuli. However, negative regulation of inflammasome activity is not well understood. Glucocorticoids (GC) are drugs of choice for the treatment of many inflammatory diseases. Recently, we could show that treatment of monocytes with GC leads to re-programming towards a specific population involved in resolution of inflammation. Gene analysis has shown up-regulated expression of 12/15-lipoxygenase (12/15-LOX) in GC-and LPS/GC-treated monocytes. 12/15-LOX reacts with polyunsaturated-fatty-acids to generate anti-inflammatory lipid-mediators, which contribute to resolution of inflammation. The aim of our study was to determine the contribution of 12/15-LOX on the inflammatory response on murine monocytes.
Materials/Methods: Bone marrow-derived monocytes were isolated from wild-type (wt) C57BL/6 and 12/15-LOX-/- mice and stimulated with GC and/or LPS as well as various inhibitors or stimulants. Gene expression was analyzed using qRT-PCR. Protein expression was examined by Western-Blot, Flow-Cytometry and ELISA. T-cell response was analyzed by co-culture of stimulated monocytes with allogenic T-cells.
Results: 12/15-LOX-/- monocytes showed slightly higher secretion of IL-1β as compared to wt cells after LPS stimulation. The differences between wt and 12/15-LOX-/- were much more pronounced when monocytes were additionally exposed to ATP. LPS treatment markedly enhanced expression of pro-IL-1β in 12/15-LOX-/- monocytes. No differences could be observed between wt and 12/15-LOX-/- monocytes in secretion of other proinflammatory mediators as well as the expression of inflammasome components. However, expression of cleaved caspase-11 was up-regulated in 12/15-LOX-/- monocytes exposed to LPS. Additionally, inhibition of caspase-11, caspase-1 and 5-LOX significantly reduced the high secretion of IL-1β in 12/15 LOX-/- monocytes. Interestingly, 12/15-LOX-/- rather than wt monocytes stimulated with LPS led to enhanced T-cell proliferation.
Conclusion: Our results demonstrate that 12/15-LOX plays a regulatory role during inflammatory immune response by counteracting the NLRP3 inflammasome activity through down-regulation of caspase-11 and 5-LOX activity. Thus, we identified a novel negative regulatory pathway of inflammasome activity.

Primary author: Dr KUSCHE, Yvonne (Institute of Immunology)

Co-authors: Prof. ROTH, Johannes (Institute of Immunology); Dr BARCZYK-KAHLERT, Katarzyna (Institute of Immunology)

Presenter: Dr KUSCHE, Yvonne (Institute of Immunology)

Contribution ID: **15** Type: **Poster**

Characterization of pericytes during autoreactive immune cell invasion into the CNS

The blood-brain barrier (BBB) is a major regulator of the central nervous system (CNS) homeostasis. In numerous neurological disorders the impairment of the BBB leads to the entry of immune cells and molecules into the CNS causes neuronal dysfunction and degeneration. Although there is a growing interest in understanding the role of pericytes as component of the BBB, little is known about their impact in CNS autoimmunity. In this project we aim to elucidate the involvement of pericytes in maintenance of the BBB and modulation of immune cell invasion during CNS inflammation. To address this, we performed in vitro and in vivo experiments with an inducible pericyte-specific Cre mouse strain. As in vivo model for (CNS) inflammation we performed LPS injections and experimental autoimmune encephalitis (EAE) experiments. Flow cytometric analysis of in vivo experiments exhibited a modulation of adhesion and antigen-presenting molecules upon inflammation. In vitro assays revealed a capacity of brain pericytes to present antigens towards T cells and promote T cell adhesion. Reciprocally, an influence of immune cells on pericyte morphology and activity could be demonstrated. In conclusion, we propose that brain pericytes may modulate CNS inflammation by direct interaction with invading T cells.

Primary author: Mrs KOCH, Kathrin (Clinic of Neurology with Institute of Translational Neurology, University Hospital of Muenster, Muenster, Germany)

Co-authors: Dr LINDNER, Maren (Clinic of Neurology with Institute of Translational Neurology, University Hospital of Muenster, Muenster, Germany); Dr DIEGUEZ-HURTADO, Rodrigo (Department of Tissue Morphogenesis, Max-Planck-Institute for Molecular Biomedicine, and Faculty of Medicine, University of Muenster); Prof. ADAMS, Ralf H. (Department of Tissue Morphogenesis, Max-Planck-Institute for Molecular Biomedicine, and Faculty of Medicine, University of Muenster); Prof. WIENDL, Heinz (Clinic of Neurology with Institute of Translational Neurology, University Hospital of Muenster, Muenster, Germany); Prof. KLOTZ, Luisa (Clinic of Neurology with Institute of Translational Neurology, University Hospital of Muenster, Muenster, Germany)

Presenter: Mrs KOCH, Kathrin (Clinic of Neurology with Institute of Translational Neurology, University Hospital of Muenster, Muenster, Germany)

Contribution ID: **16** Type: **Poster**

Mechanism and consequences of interactions between immune cells and endothelial cells at the blood-brain-barrier - implications for CNS inflammatory diseases

A common feature of inflammatory CNS diseases such as multiple sclerosis, rasmussen encephalitis, neuromyelitis optica or the Susac syndrome represents the transmigration of autoreactive T cells through the brain endothelial cell layer into the CNS, where they can cause tissue destruction and neuronal loss.
With this project we are aiming to investigate the molecular mechanisms of immune cell and endothelial cell interaction in different CNS inflammatory diseases. While previous studies mainly focused on immune cell changes, we are examining the consequences on endothelial cells due to the interaction with CD8+ T cells. While above mentioned diseases are presumably antigen driven, however how and where T cells get (re)-activated in close proximity to the CNS is not quite clear. Our data suggest that primary mouse brain microvascular endothelial cells (MBMECs) can present antigen on their surface via the MHC class I receptor and therefore are able to activate naïve antigen specific T cells. Coculture of naïve OT1 T cells with SIINFEKL loaded endothelial cells results in a rapid reduction in endothelial barrier function as illustrated by a reduced transendothelial electrical resistance (TEER) and increased permeability to soluble molecules.
The antigen specific interaction of endothelial cells with T cells caused an upregulation of MHC class I expression as well as an increase in key adhesion molecules such as ICAM and VCAM on the endothelial cell side. In order to cause endothelial cell damage a direct cell – cell contact seems necessary. Despite strong impairment of EC barrier integrity, T cells did not elicit apoptosis in endothelial cells.
Taken together, these data could identify novel antigen-dependent reciprocal interactions between brain endothelial cells and T cells with future implications for CNS inflammatory diseases.

Primary author: Mrs PLATE, Henrike Charlotte (Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany)

Co-authors: Dr LINDNER, Maren (Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany); Dr GHELMAN, Julia (Institute for Neuropathology, University of Münster, Münster, Germany); Prof. KUHLMANN, Tanja (Institute for Neuropathology, University of Münster, Münster, Germany); Prof. WIENDL, Heinz (Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany); Prof. KLOTZ, Luisa (Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany)

Presenters: Mrs PLATE, Henrike Charlotte (Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany); Prof. KLOTZ, Luisa (Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany)

Contribution ID: **17** Type: **Poster**

The role of S100A8 and S100A9 in murine keratinocytes

Introduction: S100A8 and S100A9, also known as myeloid-related protein-8 (MRP8) and MRP14, are damage-associated molecular pattern molecules (DAMPs). Both proteins are highly up-regulated in autoimmune diseases of human skin like psoriasis as well as in certain in vivo models (e.g. Imiquimod induced psoriasis like phenotype).
Methods: Keratinocytes collected from adult tail skin of either wildtype or S100A9-/- mice were differentiated under high calcium conditions (1 mM CaCl2). Furthermore keratinocytes of both mice strains were stimulated with different inflammatory mediators. Cells were harvested at different time points and analysis of gene or protein expression was performed.
Results: Both, wildtype and S100A9-/- cells, show a typical expression pattern of genes responsible for differentiation. During differentiation an increased gene expression of both S100 proteins could be detected in wildtype but not in S100A9-/- (here only S100A8) cells. On protein level S100A9-/- cells show no expression of S100A8 and S100A9 whereas wildtype cells express both proteins in increasing amounts during differentiation. Wildtype keratinocytes stimulated with IL-1α, IL-17A, IL-17F, TNFα and Flagellin show a significant elevation of S100A8 and S100A9 on mRNA and protein level compared to unstimulated or IL-13 treated cells.
Conclusion: Both wildtype and S100A9-/- keratinocytes show the same gene expression pattern of proteins known for differentiation, indicating no direct correlation of S100 proteins and keratinocyte differentiation. Surprisingly S100A9-/- keratinocytes do not express S100A8 although mRNA level were increasing during differentiation and stimulation with inflammatory mediators. These results indicate an inflammation and differentiation dependent mechanism of S100 protein expression.

Primary author: Dr CAROLIN, Weise (Institute of Immunology, University of Münster)

Co-authors: Mrs JANINA, Latzel (Institute of Immunology, University of Münster); Prof. LOSER, Karin (Department of Dermatology, University of Münster); Prof. ROTH, Johannes (Institute of Immunology, University of Münster)

Presenter: Dr CAROLIN, Weise (Institute of Immunology, University of Münster)

Contribution ID: **18** Type: **Poster**

Dimethyl fumarate treatment impairs T cell metabolism in multiple sclerosis patients

Dimethyl fumerate (DMF) is an approved immune-modulatory drug for treatment of relapsing remitting multiple sclerosis (RRMS). Although DMF treatment leads to beneficial clinical effects, around 17% of patients develop a lymphopenia characterized by sustained decrease of lymphocyte counts within blood. In parallel, DMF-treated patients exhibited decreased frequencies of memory T cell (TM) while naïve T cells (TN) were less affected. Interestingly, it is known that TM cells reveal an increased metabolic capacity compared to TN cells, at least in the murine system.
The aim of this study was to investigate whether there is a DMF-mediated link between the T cell metabolism of different T cell subsets (TM and TN) and their T cell apoptosis. T cell metabolism and mitochondrial stress response was assessed using the Seahorse technology and fluorescent protein–based redox sensors, respectively. To determine apoptotic processes in T cells we performed flow-cytometric analysis of caspases 3 and 7.
T cells, which were isolated from peripheral blood mononuclear cells from MS patients before and during treatment with DMF, displayed a decreased T cell metabolism following DMF treatment. In vitro, DMF treatment of T cells isolated from healthy donors caused a diminished mitochondrial respiration of 75% in both T cell subsets (TM and TN cells), whereby TM cells displayed a 2-fold increased metabolic capacity compared to TN cells. In this line, we determined an enhanced mitochondrial stress response exhibited in evaluated ROS levels in DMF-treated TM cells, which strongly supports the idea that TM cells display an enhanced sensitivity upon DMF treatment. Correspondingly, DMF treatment revealed a higher apoptosis rate in TM cells than TN cells.
Collectively, these data illustrate that DMF treatment significantly reduces mitochondrial respiration in all T cell subsets. Importantly, TM cells display an enhanced sensitivity and stress response following DMF treatment, which might be a potential mechanisms explaining the increased apoptosis and vulnerability of TM cells in DMF-treated MS patients.

Primary authors: Mrs LIEBMANN, Marie (Clinic of Neurology with Institute of Translational Neurology, University Hospital of Muenster, Muenster); Mrs JANOSCHKA, Claudia (Clinic of Neurology with Institute of Translational Neurology, University Hospital of Muenster); Dr HERRMANN, Alexander M. (Clinic of Neurology with Institute of Translational Neurology, University Hospital of Muenster); Dr SCHULTE-MECKLENBECK, Andreas (Clinic of Neurology with Institute of Translational Neurology, University Hospital of Muenster); Prof. SCHWAB, Nicholas (Clinic of Neurology with Institute of Translational Neurology, University Hospital of Muenster); Dr GROSS, Catharina C. (Clinic of Neurology with Institute of Translational Neurology, University Hospital of Muenster); Prof. MEUTH, Sven G. (Clinic of Neurology with Institute of Translational Neurology, University Hospital of Muenster); Prof. WIENDL, Heinz (Clinic of Neurology with Institute of Translational Neurology, University Hospital of Muenster); Prof. KLOTZ, Luisa (Clinic of Neurology with Institute of Translational Neurology, University Hospital of Muenster)

Presenter: Mrs LIEBMANN, Marie (Clinic of Neurology with Institute of Translational Neurology, University Hospital of Muenster, Muenster)

Contribution ID: **19** Type: **Poster**

Role of fibronectin and serine proteases in S. aureus invasion into host cells

Staphylococcus aureus is not only an extracellular but also an intracellular pathogen. The host cell invasion of non-professional phagocytes essentially contributes to infection development. The fibronectin bridging between *S. aureus* FnBPs and alpha5beta1 integrins on the host cell side that facilitates the uptake of staphylococci is well investigated. In a recent in vitro study we showed that typical barrier cells such as endothelial and epithelial cells were highly invaded by bacteria, whereas primary human osteoblasts and fibroblasts took up *S. aureus* to a much lesser extent [1]. To find an explanation for the differences in the uptake ability of the cells we analyzed the alpha5beta1 integrin expression by antibodies using flow cytometry. Fibronectin distribution as well as bacterial binding and uptake was analyzed by fluorescence microscopy. The number of internalized viable bacteria was determined using the lysostaphin protection assay. We used different protease inhibitors to analyze the role of proteases in bacterial uptake
Contrary to our expectations we detected high amounts of alpha5beta1 integrin on the surface of primary osteoblasts and fibroblast cell line cells, whereas endothelial and epithelial cell line cells had only a low integrin expression. Analysis of fibronectin distribution revealed low amounts of fibronectin on endothelial and epithelial cell line cells, whereas primary osteoblasts and fibroblasts were covered with thick fibronectin fibrils. *S. aureus* bound to these fibrils and proliferated there, but was not taken up into the host cells. Therefore, the fibronectin matrix fibrils around osteoblasts and fibroblasts form a mechanistic barrier against *S. aureus*. In contrast, the fibronectin on the analyzed endothelial and epithelial cells acts as the well investigated bridge builder between *S. aureus* and host cells. Microscopic analysis of bacterial uptake revealed low to high degradation of fibronectin fibrils, depending on the host cell type. This degradation as well as bacterial uptake could be blocked by serine protease inhibitors. Infection of host cells with a *S. aureus* protease knock out mutant did not lead to a reduction in fibronectin degradation; therefore, it can be assumed that serine proteases released by the host cell promote bacterial uptake.
Our results shed new light on the processes involved in the uptake of *S. aureus* into host cells.

Reference:
[1] Strobel, M., Pförtner, H., Tuchscherr, L., Völker, U., Schmidt, F., Kramko, N., Schnittler, H. J., Fraunholz, M. J., Löffler, B., Peters, G., and Niemann, S. (2016) Post-invasion events after infection with Staphylococcus aureus are strongly dependent on both the host cell type and the infecting S. aureus strain. Clin Microbiol Infect 22, 799-809

Primary author: Dr NIEMANN, Silke (Institute od Medical Microbiology, University Hospital of Münster, Münster)

Co-authors: Prof. EBLE, Johannes (Institute of Physiological Chemistry and Pathobiochemistry, University of Münster, Münster); Prof. LÖFFLER, Bettina (Institute of Medical Microbiology, Jena University Hospital, Jena); Dr VAN DE VYVER, Hélène (Institute of Medical Microbiology, University Hospital of Münster, Münster); Prof. PETERS, Georg (Institute of Medical Microbiology, University Hospital of Münster, Münster)

Presenter: Dr NIEMANN, Silke (Institute od Medical Microbiology, University Hospital of Münster, Münster)

Contribution ID: **20** Type: **Poster**

DAMP-induced proinflammatory mechanisms affect the alveolar epithelial barriers during acute lung injury (ALI)

Acute lung injury (ALI) is a heterogeneous lung injury characterised by infiltration of phagocytes in the lungs. Alveolar epithelial cells (AECs) are an essential part of the respiratory barrier in lungs for gas exchange and protection against pathogens. During ALI the alveolar capillary barrier, which is made of the epithelial layer and the endothelial layer, is disrupted. Endogenous Damage Associated Molecular Pattern Molecules (DAMPs) and Pathogen Associated Molecular Pattern molecules (PAMPs) activate the epithelial and endothelial cells to release cytokines and chemokines leading to ALI. However, their interplay as well as the mechanism of AECs' activation especially by the alarmin S100A8 is unknown. During the barrier breakdown phagocytes migrate into the lung to eliminate the infection. Thus, our aim was to study the mechanism of activation of AECs (type I and type II) by S100A8 or lipopolysaccharide (LPS) and to understand the role of endogenous S100A8 in phagocytes recruitment in the lung. We here clearly demonstrate that AEC s are activated by S100A8 via a TLR4-dependent pathway whereas RAGE, albeit mainly expressed in lung tissue, does not play a role. Additionally, we show that S100A8 is an essential factor for neutrophil recruitment to lungs and the stimulation of all the recruited phagocytes.

Primary author: Mrs RIETKÖTTER, Anna

Co-author: Dr CHAKRABORTY, Deblina

Presenter: Mrs RIETKÖTTER, Anna

Contribution ID: **21** Type: **Poster**

Hypersecretion of S100A8/A9 in non-classical autoinflammatory diseases

Introduction: Hereditary autoinflammatory diseases (AID) are a group devastating disorders characterized by chronic inflammation and unprovoked activation of phagocytes. Classical AIDs are caused by mutations in the NLRP3-inflammasome, leading to excess of IL-1β-release, whereas inflammation in non-classical AID (ncAID), e. g. PAPA- (Pyogenic sterile Arthritis, Pyoderma gangrenosum, Acne) and PAMI- (PSTPIP1-Associated Myeloid-related-Proteinaemia Inflammatory) syndrome has been linked to mutations in the PSTPIP1 and Pyrin genes and to an extraordinary overexpression of S100A8 and S100A9 in phagocytes. S100A8/A9 can be released by tubulin-dependent alternative secretory pathways to induce pro-inflammatory effects through interaction with Toll-like receptor 4 (TLR-4).
Objectives: The aim of the present project is to analyze the mechanisms in which ncAID associated PSTPlP1 mutations lead to an exaggerated S100 secretion.
Materials & methods: Monocytes of PAMI patients were isolated, and S100A8/A9 levels were measured in culture supernatants prior and after activation. PSTPIP1-Pyrin-S100A8/A9 interactions were investigated by co-immunoprecipitation, immunofluorescence- and ELISA studies.
Results: Our studies demonstrate that PSTPIP1 and pyrin interact with the S100A8-S100A9 complex. Moreover in PSTPIP1 we could map the respective binding site to the CC domain bearing disease associated mutations. Monocytes from patients carrying PSTPIP1 mutations showed an exaggerated release of S100A8/A9.
Conclusion: S100A8/A9 are highly oversecreted in FMF-, PAPA- and PAMI patients. Pyrin and PSTPIP1 interact directly with S100A8 and S100A9 pointing towards an important role of them in the alternative secretion of those proteins. Moreover PSTPIP1 mutations found in all patients are restricted to the S00A8/A9 binding region and these PSTPIP1 mutations also influence the interaction with pyrin. Overall, our data indicate that hypersecretion of S100A8/A9 is a relevant pathomechanism in PSTPIP1- and pyrin-associated diseases.

Primary authors: Dr AUSTERMANN, Judith (Institute of Immunology, University Hospital Muenster, Muenster, Germany); Dr JORCH, Selina K. (Department of Physiology and Pharmacology, Cumming School of Medicine, University of Calgary, Calgary, AB T2N 4N1, Canada); Dr HOLZINGER, Dirk (Department of Pediatrics III, University Hospital Essen, Essen); Prof. VOGL, Thomas (Institute of Immunology, University Hospital Muenster, Muenster, Germany); Prof. ROTH, Johannes (Institute of Immunology, University Hospital Muenster, Muenster, Germany)

Presenter: Dr AUSTERMANN, Judith (Institute of Immunology, University Hospital Muenster, Muenster, Germany)

Contribution ID: **22** Type: **Talk**

Dietary supplementation with conjugated linoleic acid influences CNS autoimmunity via the gut-brain axis

Multiple sclerosis (MS) is a multifactorial disorder, thus genetic disposition but also environmental factors are known to influence disease susceptibility and activity. This environmental modulation of central nervous system (CNS) autoimmunity can, *inter alia*, be mediated by the gut-brain axis, which is affected by nutritional components. Previous studies describe an influence of the poly-unsaturated fatty acid conjugated linoleic acid (CLA) in organ-specific autoimmunity such as rheumatoid arthritis and inflammatory bowel disease.

Hence, we want to characterize the immune-modulatory effects of dietary supplementation with CLA on the gut-brain axis in the context of MS. For this purpose, mice that spontaneously develop CNS autoimmunity were supplemented with CLA-rich diet. These mice showed an amelioration of disease severity accompanied by reduced CNS inflammation. Via flow cytometry, we characterized the effects on the gut-brain axis in more detail and determined changes in intestinal immune cell responses of CLA-fed mice, such as increased frequencies of regulatory T cells and affected myeloid cell activity in the intestine. Real-time measurement of oxygen consumption and extracellular acidification rate revealed an impaired energy metabolism in various immune cell subsets after CLA treatment *in vitro*. Besides direct effects on immune cells, CLA-rich diet alternated the intestinal microbiome composition, which can further modulate the gut-brain axis. Lactobacillus and Clostridium species are increased, whereas Prevotella and *Blautia* are reduced, which was contrary to the described microbiome of MS patients. To translate these findings into the human system, we performed a pilot study with 15 MS patients, which were daily supplemented with CLA over a period of six months. Similar to murine results, we determined immune-modulatory and some microbiota-changing effects in MS patients after CLA supplementation.

These data illustrate that dietary factors, such as CLA, play an important role in the modulation of CNS autoimmunity and further reflect a therapeutic potential to improve these autoimmune processes via the gut-brain axis.

Primary author: FLECK, Ann-Katrin (Department of Neurology with Institute of Translational Neurology, University Hospital of Münster)

Co-authors: HUCKE, Stephanie (Department of Neurology with Institute of Translational Neurology, University Hospital of Münster); HARTWIG, Marvin (Department of Neurology with Institute of Translational Neurology, University Hospital of Münster); TEIPEL, Flavio (Department of Neurology with Institute of Translational Neurology, University Hospital of Münster); HEROLD, Martin (Department of Neurology with Institute of Translational Neurology, University Hospital of Münster); BERER, Kerstin (Department of Neuroimmunology, Max-Planck Institute of Neurobiology, Martinsried); LIEBMANN, Marie (Department of Neurology with Institute of Translational Neurology, University Hospital of Münster); KUZMANOV, Ivan (Department of Neurology with Institute of Translational Neurology, University Hospital of Münster); GRÜTZKE, Berit (Department of Neurology with Institute of Translational Neurology, University Hospital of Münster); EVESLAGE, Maria (Institute of Biostatistics and Clinical Research, University of Münster); GROSS, Catharina C. (Department of Neurology with Institute of Translational Neurology, University Hospital of Münster); KRISHNAMOORTHY, Gurumoorthy (Department of Neuroimmunology, Max-Planck Institute of Neurobiology, Martinsried); DOBRINDT, Ulrich (Institute of Hygiene, University Hospital of Münster); SCHUPPAN, Detlef (Institute of Transla-

tional Immunology, University of Mainz); KUHLMANN, Tanja (Institute of Neuropathology, University of Münster); WIENDL, Heinz (Department of Neurology with Institute of Translational Neurology, University Hospital of Münster); KLOTZ, Luisa (Department of Neurology with Institute of Translational Neurology, University Hospital of Münster)

Presenter: FLECK, Ann-Katrin (Department of Neurology with Institute of Translational Neurology, University Hospital of Münster)

Contribution ID: **23** Type: **Talk**

Detecting bacteria-induced infective endocarditis by elemental mass spectrometric imaging

Staphylococcus aureus-induced infective endocarditis (IE) is a life threatening disease. To investigate and characterize IE in vivo, we have recently established a mouse model [1], [2]. IE is induced by placing a permanent catheter into the right carotid artery to irritate the aortic valves and provide a seed for formation of bacterial vegetations. Pathology of IE accumulates different amounts of chemical elements. The competition for metals between host and pathogen is one of the most important factors dictating the outcome of infection. Here, we assess whether mass spectrometry can identify IE.

A 32G-catheter was placed on the aortic valves for 48 h. Next, 24 h after catheter placement, C57Bl/6 mice were infected with S. aureus or PBS (MOCK-infected). Mice were sacrificed and the hearts were embedded in paraffin. Aortic valves were sliced 10 µm thick to perform laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to determine the amount of Ca, Cu, Fe, Mg, , Zn, and Mn. A qualitative measurement was done for P. Consecutive tissue slices were stained for Gram or H&E to relate element concentrations to infection or inflammation.

IE was successfully induced as confirmed by Gram staining that revealed S. aureus vegetations on the aortic valves. Focusing on this infected tissue, LA-ICP-MS showed a high concentration of Ca (maximum value: 800 µg/g) and a high qualitative intensity of P. High amount of Zn (maximum value: 250 µg/g) was detected where histology suggested an increased presence of immune cells. Increased Mn (maximum value: 4 µg/g) was observed where histology showed the presence of osteoblasts.

Calcium, phosphor and zinc in combination may be used to identify bacterial vegetations in IE. LA-ICP-MS may become a valuable tool to differentiate between calcification and infection.

References:
[1] Ring J et al. (2014). PLoS ONE 9(9): e107179. [2] Hoerr V et al. (2013). J Cardiovasc Magn Reson.15:59.

Primary authors: Mr SCHWARZ, Christian (Department of Clinical Radiology, Translational Research Imaging Center (TRIC), University Hospital Muenster); Mrs BUCHHOLZ, Rebecca (Institute of Inorganic and Analytical Chemistry, Westfalian Wilhelms University Muenster); Prof. KAST, Uwe (Institute of Inorganic and Analytical Chemistry, Westfalian Wilhelms University Muenster); Prof. WILDGRUBER, Moritz (Department of Clinical Radiology, Translational Research Imaging Center (TRIC), University Hospital Muenster); Prof. FABER, Cornelius (Department of Clinical Radiology, Translational Research Imaging Center (TRIC), University Hospital Muenster)

Presenter: Mr SCHWARZ, Christian (Department of Clinical Radiology, Translational Research Imaging Center (TRIC), University Hospital Muenster)

Contribution ID: **24** Type: **Poster**

Analysis of regulation of S100A8/A9-expression in phagocytes via CRISPR/Cas9 genome editing

The alarmins S100A8 and S100A9 are important signaling molecules during the innate immune response of monocytes and neutrophils. Indeed, S100A8 and S100A9 are dys-regulated in numerous tumors and inflammatory diseases. To gain insights into mechanisms of transcriptional regulation of S100A8/A9, four candidate transcription factors were tested for their impact on S100A8/A9 expression in murine monocytes and granulocytes obtained from transiently immortalized myeloid stem cells (ER-Hoxb8 cells). The recent CRISPR/Cas9 genome editing system that is able to precisely cleave desired gene locations was used to generate candidate transcription-factor specific-knock-out ER-Hoxb8 cell lines. Further knock-out cell lines were obtained by generating ER-Hoxb8 cells of bone marrow derived cells from transgenic mice. Analysis of S100A8/A9 expression during differentiation of knock-out cell lines identified, inter alia, ATF3 as a potential negative regulator of these alarmins in monocytes. Interestingly, the bile acid receptor FXR was suggested to be a positive regulator of S100A8/A9 in this study in both, monocytes and neutrophils. Analysis also revealed C/EBPδ as a positive regulator of S100A8/A9 in ER-Hoxb8 neutrophils. Through these findings we took a step forward in understanding mechanisms behind transcriptional regulation of S100A8 and S100A9.

Primary author: Mrs JAUCH, Saskia-L. (Institut für Immunologie)

Presenter: Mrs JAUCH, Saskia-L. (Institut für Immunologie)

Contribution ID: **26** Type: **Poster**

FRET-based MMP14 probes for the investigation of invadopodia of invasive cancer cells

Metastatic tumor cells breach the barriers posed by the basement membrane, the interstitial connective tissue and the blood vessel wall in the so-called metastatic cascade. For invasion, both adhesion to the extracellular matrix and a spatially matched proteolysis of the extracellular matrix are essential. Therefore, invasive cancer cells form actin-rich protrusions on which β1 integrins and MMP14 are coupled both structurally and functionally to allow a tumor cell to adhere to and also to degrade extracellular matrix molecules. Hence, a combined inhibition of adhesion and/or protease activity may reduce the invasive properties of tumor cells. In order to analyze the MMP14 activity in invadopodia, Förster resonance energy transfer (FRET) probes are generated. Bifunctional compounds with specific integrin-and MMP14-targeting domains are designed to elucidate the structural and functional link and to suppress the barrier-breaching invadopodia activity.

Primary authors: Dr NILAND, Stephan (Institute of Physiological Chemistry and Pathobiochemistry, Münster University Hospital); Prof. EBLE, Johannes A. (Institute of Physiological Chemistry and Pathobiochemistry, Münster University Hospital)

Presenter: Dr NILAND, Stephan (Institute of Physiological Chemistry and Pathobiochemistry, Münster University Hospital)

Contribution ID: **27** Type: **Poster**

Time-lapse MRI as dynamic single cell tracking imaging technique for diagnosis of inflammation

Introduction:
Time-lapse MRI enables imaging of slowly moving individual immune cells in the intact brain in vivo. Applying this method to naïve mice and mice suffering from experimental autoimmune encephalomyelitis (EAE) has previously shown altered immune cell dynamics. The aim of this study is to evaluate time-lapse MRI of the brain under non-CNS associated inflammatory conditions.

Methods:
In a first animal model, C57BL/6 mice were injected subcutaneously with 100µl of a polyacrylamide-gel (pellet) containing lipopolysaccharide (LPS, 10, 20, 40µg LPS/100µl PAG) to induce a sterile local inflammation (n=6 each). After 24h mice were i.v. injected with iron oxide nano particles (Resovist, Ferucarbotran, 1.9ml/kg BW). 24h later time-lapse MRI of the whole brain with 20 time-frames (scan time: 8min 12s for a single time-frame) was performed.
In a second model C57BL/6 mice (n=3) were i.v. injected with 1x10⁵ CFU of S. aureus, followed by the same time-lapse MRI protocol.
Healthy C57BL/6 mice (n=8) injected only with Resovist were used as control.
Data was analyzed manually by counting labelled cells in the brain, detected as hypointense spots (events).

Results:
Time-lapse MRI enables early detection of non-CNS associated inflammation. In control mice (269±29) events were observed, a similar number as reported previously (Masthoff et al 2018). In the local inflammation model, the number of events was significantly reduced to (173±16). In the systemic sepsis model with (19±2) events this reduction was even more pronounced.

Discussion:
As shown previously time-lapse MRI enables detection and quantification of altered immune cell dynamics between health and disease. Not only EAE as CNS autoinflammation but also local peripheral inflammation as well as systemic sepsis has altered immune cell dynamics in the brain, as detected and quantified by time-lapse MRI. Immune cell dynamics are dependent on the quality and intensity of the inflammatory stimulus.

Primary author: Mr FREPPON, Felix (1. Translational Research Imaging Center (TRIC), Department of Clinical Radiology, University Hospital Muenster, North Rhine-Westphalia, Germany)

Co-authors: Dr MASTHOFF, Max (1. Translational Research Imaging Center (TRIC), Department of Clinical Radiology, University Hospital Muenster, North Rhine-Westphalia, Germany); Dr WACHSMUTH, Lydia (1. Translational Research Imaging Center (TRIC), Department of Clinical Radiology, University Hospital Muenster, North Rhine-Westphalia, Germany); ALBERS, Franziska (1. Translational Research Imaging Center (TRIC), Department of Clinical Radiology, University Hospital Muenster, North Rhine-Westphalia, Germany); Mr SCHWARZ, Christian (1. Translational Research Imaging Center (TRIC), Department of Clinical Radiology, University Hospital Muenster, North Rhine-Westphalia, Germany); Dr GERWING, Mirjam (1. Translational Research Imaging Center (TRIC), Department of Clinical Radiology, University Hospital Muenster, North Rhine-Westphalia, Germany); Dr HELFEN, Anne (1. Translational Research Imaging Center (TRIC), Department of Clinical Radiology, University Hospital Muenster, North Rhine-Westphalia, Germany); Prof. HEINDEL, Walter (1. Translational Research Imag-

ing Center (TRIC), Department of Clinical Radiology, University Hospital Muenster, North Rhine-Westphalia, Germany); Dr EISENBLÄTTER, Michel (1. Translational Research Imaging Center (TRIC), Department of Clinical Radiology, University Hospital Muenster, North Rhine-Westphalia, Germany); Prof. WILDGRUBER, Moritz (1. Translational Research Imaging Center (TRIC), Department of Clinical Radiology, University Hospital Muenster, North Rhine-Westphalia, Germany); Prof. FABER, Cornelius (1. Translational Research Imaging Center (TRIC), Department of Clinical Radiology, University Hospital Muenster, North Rhine-Westphalia, Germany)

Presenter: Mr FREPPON, Felix (1. Translational Research Imaging Center (TRIC), Department of Clinical Radiology, University Hospital Muenster, North Rhine-Westphalia, Germany)

Contribution ID: **28** Type: **Poster**

MRI investigation of clearance mechanisms of the Glymphatic System

Introduction:
The Glymphatic System has been identified as a metabolic waste products clearance system in the brain, depending on paravascular pathways and the flux of cerebrospinal fluid (CSF). CSF flow elutes soluble proteins and waste, and is intensified during sleep or anesthesia. In our experiments we evaluate the effect of brain state on distribution of contrast agent and water diffusivity.
Methods:
For the measurements we used female Fisher rats and performed MRI under three different anesthetic regimens (Isoflurane (Iso), Medetomidine (Mede) and Iso+Mede) to resolve potential differences in glymphatic clearance originating from different brain states, which were monitored by optical calcium recordings. As contrast agent we use Gadovist, delivered by a modified venous catheter implanted into the Cisterna magna. Apparent diffusion coefficient (ADC) maps and T1 weighted 3D FLASH MRI were acquired during 6 hours. Manually drawn ROIs in CSF-compartments and brain parenchyma were analyzed.
Results:
Signal changes in T1w MRI, originating from distribution of contrast agent, which is indicative of glymphatic activity, were dependent on the anesthetic regimen. Higher signal increase in subarachnoidal space and in cortex and brain stem parenchyma was observed under Mede compared to Iso. Under Mede a significantly lower ADC was observed as compared to Iso. The combination of Iso+Mede, although reproducing a sleep-like brain state as under Iso alone, resulted in high T1w signal (similar to Mede), but low ADC in cortex (similar to Iso).
Discussion:
Our data confirm the assumption that different brain states, induced by different anesthetics, have impact on glymphatic waste clearance. They also suggest a strong influence of CSF production, which is increased under Mede and Iso + Mede. ADC maps support the notion that the lowered diffusion under awake-like brain state (Mede) is possibly caused by an increase of cellular volume of activated neurons and therefore a reduced water volume in the ECM. Those results indicate a less efficient glymphatic clearance in awake-like brain state. Further investigation into the exact pathways of glymphatic clearance under different anesthetic regimens or pharmacological modulation is therefore warranted.

Primary author: Mr GAGEL, Matthias (Translational Research Imaging Center (Tric), Department of clinical radiology, university hospital muenster, Germany)

Co-authors: Mr SEGEROTH, Martin (Translational Research Imaging Center (Tric), Department of clinical radiology, university hospital muenster, Germany); Dr WACHSMUTH, Lydia (Translational Research Imaging Center (Tric), Department of clinical radiology, university hospital muenster, Germany); Prof. FABER, Cornelius (Translational Research Imaging Center (Tric), Department of clinical radiology, university hospital muenster, Germany)

Presenter: Mr GAGEL, Matthias (Translational Research Imaging Center (Tric), Department of clinical radiology, university hospital muenster, Germany)

Contribution ID: **30** Type: **Poster**

Pathogenic signature of Th17 cells enhances during long-term natalizumab treatment – potential implications for disease rebound?

A multiple sclerosis (MS) treatment is natalizumab (NAT; Tysabri®), a monoclonal antibody against the cell adhesion molecule very late antigen-4 (VLA-4), which prevents leukocytes to adhere to the blood-brain-barrier (BBB) and therefore invasion into the central nervous system (CNS). However, after NAT cessation, MS patients frequently experience a disease rebound, often exceeding pretreatment levels of disease activity. It has been suggested that the pathophysiology of this rebound syndrome may be linked to interleukin (IL)-17 producing T helper (Th17) cells.
We investigated by multi-colour flow cytometry and transcriptome analysis the impact of NAT treatment on the cytokine profile and pathogenic gene expression signature of Th17 cells in relapsing-remitting MS patients. Moreover, we evaluated the functional characteristics of Th17 cells on human brain endothelial cell barrier properties as well as human oligodendrocyte survival in co-culture assays. To corroborate these findings and proof-of-concept, we performed an immunohistochemical analysis of brain histology material of a NAT rebound patient.
Under long-term NAT treatment, Th17 cells acquired a pronounced pathogenic profile as illustrated by significantly increased production of proinflammatory cytokines such as IL-17A, IL-22 and GM-CSF. Furthermore, these Th17 cells exhibited transcriptional network changes associated with enhanced pathogenicity. In an in vitro model mirroring the brain endothelial barrier properties we observed that Th17 cells, in contrast to other CD4 T cell subsets, acquire an increased potential for endothelial barrier disruption during the course of NAT treatment. In this line, we observed a significant increase in Th17 cells in the cerebrospinal fluid under NAT compared to naïve MS patients, suggesting preferential egress of Th17 cells into the CNS. Furthermore, Th17 cells but not other CD4 T cells from NAT patients exhibited an enhanced capacity to kill oligodendrocytes. Finally, histology of a biopsy specimen from a NAT rebound patient revealed a striking predominance of Th17 cells within an inflammatory lesion, hence corroborating the relevance of Th17 cells in the pathogenesis of disease rebound.
Taken together our data suggest that long-term NAT treatment is associated with an acquisition of increased pathogenicity of Th17 cells, which might explain the clinical phenomenon of enhanced disease activity upon NAT cessation.

Primary authors: Mrs JANOSCHKA, Claudia (Department of Neurology with Institute of Translational Neurology, University Hospital Münster); Prof. WIENDL, Heinz (Department of Neurology with Institute of Translational Neurology, University Hospital Münster); Prof. KLOTZ, Luisa (Department of Neurology with Institute of Translational Neurology, University Hospital Münster)

Co-authors: Dr SCHULTE-MECKLENBECK, Andreas (Department of Neurology with Institute of Translational Neurology, University Hospital Münster); Mrs STAROST, Laura (Institute for Neuropathology, University of Münster); Mr SCHAFFLICK, David (Department of Neurology with Institute of Translational Neurology, University Hospital Münster); Dr SCHNEIDER-HOHENDORF, Tilman (Department of Neurology with Institute of Translational Neurology, University Hospital Münster); Dr MEYER ZU HÖRSTE, Gerd (Department of Neurology with Institute of Translational Neurology, University Hospital Münster); Dr GROSS, Catharina C. (Department of Neurology with Institute of Transla-

tional Neurology, University Hospital Münster); Prof. SCHWAB, Nicholas (Department of Neurology with Institute of Translational Neurology, University Hospital Münster); Prof. KUHLMANN, Tanja (Institute for Neuropathology, University of Münster, Muenster)

Presenter: Mrs JANOSCHKA, Claudia (Department of Neurology with Institute of Translational Neurology, University Hospital Münster)

Contribution ID: **31** Type: **Poster**

Are B cells attracted by T follicular helper cells in experimental central nervous system autoimmunity?

Maike Hartlehnert (1), David Schafflick (1), Tobias Lautwein (1), Jan-Kolja Strecker (1), Tanja Kuhlmann (2), Heinz Wiendl (1), Gerd Meyer zu Horste (1)
(1) Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany; (2) Institute of Neuropathology, University Hospital Münster, Münster, Germany.

T follicular helper (TFH) cells are a CD4+ T cell subset homing to germinal centres (GCs) of secondary lymphoid organs. Here, TFH cells play a crucial role during the maturation of B lineage cells in physiological immune responses. Pathological structures, morphologically similar to GCs called ectopic lymphoid tissue, can develop in the meninges of multiple sclerosis (MS) patients and correlate with MS severity.
We here speculated that TFH cells interact with B cells in a 'pathological GC reaction' locally in the meninges and may promote MS pathology.
We induced the experimental autoimmune encephalomyelitis (EAE) animal model of MS in TFH cell-deficient (CD4CreBcl6flox/flox) mice and found the severity of MOG35-55 peptide induced EAE decreased compared to controls. This correlated with a significantly reduced amount of pathogenic T cells and total B cells infiltrating in the central nervous system (CNS) of TFH-cell deficient mice. To further characterize these B cells in the CNS, we performed transcriptomic profiling.
We also performed an adoptive transfer (AT) EAE using MOG-specific T cells derived from TFH-cell deficient mice transgenically expressing a MOG-specific T cell receptor and their wildtype littermates. We found abundant GC-like ectopic lymphoid tissue in the spinal cord in these mice and CD4CreBcl6flox/flox myelin-reactive T cells induced less severe AT-EAE than wildtype-derived T cells. We are further characterizing ectopic lymphoid tissue to understand local T/B-cell interaction in this model.
Taken together, our data suggest that TFH cells contribute to CNS autoimmunity and exacerbate B cell infiltration in the CNS.

Primary author: Mrs HARTLEHNERT, Maike (Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany)

Co-authors: Mr SCHAFFLICK, David (Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany); Dr LAUTWEIN, Tobias (Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany); Dr STRECKER, Jan-Kolja (Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany); Prof. KUHLMANN, Tanja (Institute of Neuropathology, University Hospital Münster, Münster, Germany); Prof. WIENDL, Heinz (Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany); Prof. MEYER ZU HORSTE, Gerd (Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany)

Presenter: Mrs HARTLEHNERT, Maike (Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany)

Contribution ID: **32** Type: **Poster**

Discovery of a species-spanning family of bacteria-derived cell-penetrating effector proteins

Many pathogenic bacteria secreted effector proteins directly into the eukaryotic host cell cytoplasm through their type III secretion system (T3SS), where they exert a number of effects that enable the pathogen to survive and to escape the host defense mechanisms. Lately, we could identify the Yersinia effector YopM as well as Salmonella-derived SspH1 as the first bacteria-derived cell-penetrating effectors (CPEs) that are also able to translocate into host cells in a T3SS-independent manner. In addition to both YopM and SspH1 which belong to the bacterial effectors of the LPX subtype of leucine-rich repeat (LRR) proteins, the Salmonella proteins SspH2 and SlrP as well as different IpaH proteins of Shigella are also part of the LPX family.
Here, we investigated both the cell-penetrating abilities and the functionality of different recombinantly expressed LPX effector proteins as novel ubiquitin E3 ligases (NEL).

Primary author: Mr IVAN, Ngueya Yango (Institut für Infektiologie)

Presenter: Mr IVAN, Ngueya Yango (Institut für Infektiologie)

Contribution ID: **33** Type: **Poster**

Evaluation of the endothelial permeability of breast tumors with an albumin-binding contrast medium

Purpose: To evaluate whether the change of endothelial permeability during tumor progression can be assessed with the albumin-binding contrast medium gadofosveset in MRI.

Material and Methods: For the MRI protocol, anatomical T2, diffusion-weighted, T1/T2 map and dynamic contrast-enhanced sequences after the administration of gadofosveset trisodium were acquired. For the longitudinal study, BALB/c mice bearing a 4T1 (highly aggressive mammary carcinoma) or a 67NR (lowly malignant mammary carcinoma) were assessed by imaging, before the tumor was harvested for histology, immunofluorescence and mass spectrometry imaging.

Results: The MRI protocol has been established and 4T1 tumors have been evaluated longitudinally over nine days. Tumors show a rapid enhancement after i.v. injection of the contrast medium, the maximum is reached after about ten minutes.
Immunohistochemistry visualized a high proliferation rate, as demonstrated with positive staining for Ki67, as well as the expression of CD31 and VEGF.

Conclusion: The established MRI protocol can be used to evaluate the feasibility of measuring endothelial permeability of tumors with albumin-binding gadofosveset.

Primary author: Dr GERWING, Mirjam (University Clinic Muenster, Department of Clinical Radiology)

Co-authors: Dr WACHSMUTH, Lydia (University Hospital Muenster, Department of Clinical Radiology); Prof. EBLE, Johannes (University of Muenster, Institute of Physiological Chemistry and Pathobiochemistry); Prof. KARST, Uwe (University of Muenster, Institute of Inorganic and Analytical Chemistry); Prof. HEINDEL, Walter (University of Muenster, Department of Clinical Radiology); Prof. FABER, Cornelius (University of Muenster, Department of Clinical Radiology); Prof. WILDGRUBER, Moritz (University of Muenster, Department of Clinical Radiology)

Presenter: Dr GERWING, Mirjam (University Clinic Muenster, Department of Clinical Radiology)

Contribution ID: **36** Type: **Poster**

An Extracorporeal Circulation Mouse Model for Simultaneous Measurements of Dynamic Contrast-Enhanced Arterial Input Functions and Radiotracer Blood Concentrations

In small animals quantification of the dynamic arterial input function (AIF) is equally challenging for PET and MRI. AIF measurements provide the basis for kinetic modeling of both MR contrast agents (CA) and PET tracers.

Methodology:
Intracranial tumor-bearing mice received an extracorporeal shunt from the femoral artery to the tail vein. MRI scanning was performed using a 9.4 T MRI (Bruker BioSpec) and a cryo-cooled surface coil. The extracorporeal line featured two thickenings which resided in the MRI field of view. A MRI-compatible measuring chamber for a β-Microprobe (biospace lab) was included in the circulation. Dynamic MRI scanning of the head was performed for 15 minutes using a 3D FLASH with a spatial resolution of 0.175 x 0.175 x 1 mm and a temporal resolution of 4.015 s. A 100 µl solution containing 10-20 MBq F-18-PSMA-1007 and CA (Gadovist, 35 mM) was injected intravenously at 1 ml/min. Dispersion correction for MRI CA was performed based on the recorded distinct dispersion effect at the two interspaced thickenings. Dispersion correction for the PET tracer was performed empirically based on calibration measurements.

Results:
Our method allows for simultaneously resolving the AIF of MR CA and PET radiotracer as well as CA dynamics in tissue. The MR-based quantification shows good agreement with circulated human blood with defined CA concentrations in the range of expected concentrations and flow velocities. The CA-AIFs of 8 recorded mice show a close range of peak concentrations, little noise, typical AIF curve shape related to the simultaneously recorded PET-tracer curves.

Conclusions:
We present a novel approach for DCE-measurements of the AIF in mice providing the basis for quantitative estimation of blood-brain barrier integrity in mice. Moreover, we present the first dual recordings of AIFs of a MR CA and a PET tracer in mice. This supports evaluation approaches to deduce the CA/PET tracer AIF from one another. Further, it might provide the basis for simultaneous and integrated modeling of PET tracer and CA kinetics in mice, which is of high interest in integrated, simultaneous small animal PET/MRI.

Acknowledgment:
P.Backhaus was supported by the SFB 1009 with a 12 month “Research Rotation”.

Primary authors: BACKHAUS, Philipp (Department of Nuclear Medicine, University Hospital Münster, Münster, Germany); BÜTHER, Florian (Department of Nuclear Medicine, University Hospital Münster, Münster, Germany); WACHSMUTH, Lydia (Translational Research Imaging Center - TRIC, University of Münster, Germany); FROHWEIN, Lynn (European Institute for Molecular Imaging - EIMI, University of Münster, Germany); SCHÄFERS, Klaus (European Institute for Molecular Imaging - EIMI, University of Münster, Germany); HERMANN, Sven (European Institute for Molecular Imaging - EIMI, University of Münster, Germany); SCHÄFERS, Michael (Department of Nuclear Medicine, University Hospital Münster, Münster, Germany); FABER, Cornelius (Translational Research Imaging Center - TRIC, University of Münster, Germany)

Presenter: BACKHAUS, Philipp (Department of Nuclear Medicine, University Hospital Münster, Münster, Germany)

Contribution ID: **40** Type: **Poster**

The phagocytic activity of macrophages derived from different origins

Macrophages, as professional phagocytic cells, remove apoptotic cells during inflammation but also in routine tissue homeostasis. Additional to their defining phagocytic function, macrophages within different tissues exhibit unique properties. The mechanisms which control this phenotypic and functional heterogeneity among macrophages are currently under investigation. We studied the phagocytic activity of *in vitro* derived macrophages from spleen and bone marrow progenitors and of macrophages derived from Hoxb8 immortalized progenitors. The quantities of actively phagocytosing cells at different time points during their differentiation process were compared by microscopic means. Furthermore, the expression of general macrophage marker genes and genes specifically transcribed in phagocytosis were analyzed. We observed that the origin of the macrophages and the differentiation stage affected the gene expression and the phagocytic activity. These results of this ongoing experiment emphasize the heterogeneity among macrophages and indicate the direction for subsequent studies to further investigate the unique properties of different macrophage populations.

Primary author: LEUSSINK, Sophia (Institut für Immunolgie)

Presenter: LEUSSINK, Sophia (Institut für Immunolgie)