Swiss
Multiple Sclerosis
Society

Swiss MS Researcher Meeting 2014

Friday, September 26th, 2014

Best Western Hotel, Merian Spitz

Merian Saal, Rheingasse 2, CH-4058 Basel

ABSTRACTS

The programme and abstracts of the Swiss MS Researcher Meeting
can also be found on the website of the
Swiss MS-Society

www.multiplesklerose.ch

Swiss MS-Society, Josefstrasse 129, CH-8031 Zurich, phone +41 43 444 43 43, fax +41 43 444 43 44
info@multiplesklerose.ch, www.multiplesklerose.ch, postal account 80-8274-9
Centre romand SEP, rue du Simplon 3, CH-1006 Lausanne, phone +41 21 614 80 80, fax +41 21 614 80 81

A) TALKS

The neuroprotective hepatocyte growth factor limits the development of IL-17-producing CD8+ T cells

K. Bjarnadottir, N. Molnarfi, M. Benkhoucha, G. Schneiter, and P.H. Lalive

University of Geneva, Department of Pathology and Immunology

Background. Data indicate that interleukin (IL)-17-producing $CD8^+$ T cells (Tc17) contribute to the initiation of CNS autoimmunity in mice and humans by supporting Th17 cell pathogenicity. Thus far, our studies indicate that hepatocyte growth factor (HGF), a potent neuroprotective factor, attenuates both $CD4^+$ T cell-mediated autoimmune neuroinflammation[1, 2], and the effector function of type I cytotoxic $CD8^+$ T cells (Tc1)[3], via the development of tolerogenic dendritic cells (DCs). The immunomodulatory impact of HGF on Tc17 cell responses, however, remains to be determined.

Methods. Here we examined whether HGF affects the *de novo* differentiation of murine Tc17 cells from naïve precursors. Analogous to $CD4^+$ T cells, naïve $CD8^+$ T cells acquire an IL-17-producing phenotype in the presence of TGF-β1, IL-6 and αIFN-γ.

Results. TGF-β1, IL-6 and αIFN-γ efficiently differentiated naïve $CD8^+$ T cells into IL-$17A^+IFN\text{-}\gamma^-$ Tc17 cells. Inclusion of HGF in cell culture media, in which $CD8^+$ T cells were activated with CD28/CD3 co-ligation in the presence of other immune (splenic) cells, has a negative effect in the Tc17-promoting effect of TGF-β1 and IL-6. Similar results were observed when IL-1β alone or in combination with IL-23, two cytokines considered promoting Tc17 commitment, was supplemented to the Tc17-polarized cultures. Flow cytometric analysis of c-Met expression (the HGF receptor) by splenic cells suggests that the action of HGF is mostly indirect via DC modulation.

Conclusions. Our findings indicate that HGF treatment can reduce the development of Tc17 cells. Complementary to its impact on $CD4^+$ T cells, our findings further suggest that HGF treatment could be exploited to control $CD8^+$ T-cell-mediated autoimmune dysfunctions such as MS.

References.

1. Benkhoucha M, Santiago-Raber ML, Schneiter G, et al. Hepatocyte growth factor inhibits CNS autoimmunity by inducing tolerogenic dendritic cells and CD25+Foxp3+ regulatory T cells. Proc Natl Acad Sci U S A 2010;107:6424-6429.
2. Benkhoucha M, Molnarfi N, Dunand-Sauthier I, et al. Hepatocyte Growth Factor Limits Autoimmune Neuroinflammation via Glucocorticoid-Induced Leucine Zipper Expression in Dendritic Cells. J Immunol 2014.
3. Benkhoucha M, Molnarfi N, Schneiter G, Walker PR, Lalive PH. The neurotrophic hepatocyte growth factor attenuates CD8+ cytotoxic T-lymphocyte activity. J Neuroinflammation 2013;10:154.

A comparison of MS patients with and without cortical lesions: Demographical, cognitive and cortical thickness differences

O. Geisseler[1,2], T. Pflugshaupt[1,4], L. Bezzola[2], K. Reuter[1], B. Schuknecht[3], P. Brugger[1], M. Linnebank[1]

[1] *Department of Neurology, University Hospital Zurich*

[2] *Division of Neuropsychology, Institute of Psychology, University of Zurich*

[3] *Medizinisch Radiologisches Institut, Zurich, Switzerland*

[4] *Department of Internal Medicine, Centre of Neurology and Neurorehabilitation, Luzerner Kantonsspital*

Background. Multiple Sclerosis (MS) is classically considered a white matter disease; however, MS pathology also involves the grey matter. There is increasing evidence of an important role of cortical lesions in determining disability progression with physical and neuropsychological dysfunction.

Aim. The purpose of this study was to investigate the relevance of cortical lesions and cortical thinning in patients with relapsing remitting MS.

Methods. 48 relapsing-remitting MS patients and 48 healthy age-, education- and gender-matched controls (HC) were recruited. Structural MRI, including a double inversion recov-

ery (DIR), a T1-MPRAGE and T2-FLAIR sequence were conducted in all subjects. Cognitive functioning was assessed by an extended neuropsychological examination. In addition, all patients underwent clinical neurological examination. Lesion burden was analysed with MRI*cron*, cortical thickness with *Freesurfer*.

Results. Thirty-eight patients (79%) showed at least one cortex-involving lesion, building the CL group. The non-cortical lesion group (nCL) consisted of 10 MS patients (21%). No significant differences were observed between the two patient groups with regard to EDSS score, disease duration or age at disease onset. In the CL group the highest cortical lesion occurrence was observed bilateral in the parahippocampal gyrus. The CL group showed a significant thinner cortex than the nCL and the HC group, whereas the nCL group and the HC group did not differ. In tests assessing memory functions, the CL group scored significantly lower than the nCL group.

Conclusion. We observed that patients with cortical lesions have a thinner cortex than patients without cortical lesions, whose cortex thickness is normal, relative to healthy participants. Whether cortical thinning and the occurrence of cortical lesions result from the same pathology and whether this is different from patients without cortical involvement remains speculative. However, our data suggest that such cortical involvement plays a crucial role in the memory performance of RRMS patients.

Sterile inflammation in MS: cell surface bioactive lipids on stimulated T cells induce cytokine production in human monocytes/macrophages

Rakel Carpintero[1], Isabelle Riezman[2], Lyssia Gruaz[1], Howard Riezman[2], Danielle Burger[1]

[1] *Division of Immunology and Allergy*

[2] *Department of Biochemistry, University of Geneva*

Imbalance in cytokine homeostasis plays an important part in the pathogenesis of MS. Stimulated T cells exert a pathological effect through direct cellular contact with monocytes/macrophages, inducing a massive up-regulation of IL-1β, TNF and other inflammatory factors in the latter cells. Our previous results demonstrated that lipids extracted from plasma membranes of stimulated HUT-78 cells or stimulated peripheral blood T lymphocytes in-

duced cytokine production in human monocytes and that lipid profiles between stimulated and unstimulated cells membranes lipids were different. The aim of our work is the characterization of T cell bioactive lipids.

Total lipids extracted from membranes of HUT-78 cells were separated in different lipid classes, i.e, sterols, glycolipids and phospholipids by solid phase extraction (SPE) in SiOH column. Activation of human monocytes by lipid fractions demonstrated that the combination of glycolipids and phospholipids were required for IL-1β production in human monocytes, whereas only glycolipids were needed for sIL-1Ra production. Sterols/neutral lipids were not implicated in pro and anti-inflammatory cytokine production. Treatment of total lipids with methylamine demonstrated that sphingolipids were not involved in monocyte activation. Ones established the lipids classes implicated in cytokine production on monocytes, active fractions were collected and a second separation step was carried out in SPE-NH_2 column. Active fraction was analyzed in QExactive mass spectrometer and 49 potential bioactive lipids (m/z) were identified, whose concentrations were increased in membranes of stimulated cells compared to unstimulated cells. Mass versus charge (m/z) results obtained were not registered in lipid data base “Lipid Maps” (www.lipidmaps.org). In order to characterize these lipids, the selected m/z were fragmented with different energies and fragments were analyzed by TSQ/MRM. The results of the latter experiments are currently under analysis.

Together with our preliminary data, the present results suggest that different lipids control pro and anti-inflammatory cytokine production and that the combination of some of them are required for the induction of IL-1β in human monocytes.

Our current results demonstrate that upon stimulation, surface lipids of stimulated T cells are modified and in turn display the ability to induce cytokine production in human monocytes. The identification of these lipids might open the way to new therapeutic approaches in MS.

Motor learning in patients with multiple sclerosis: Preliminary results of an fMRI study.

Stefano Magon, Armanda Pfister, Laura Gaetano, Martin Lüethi, Athina Papadopoulou, Michael Amann, Christoph Stippich, Ernst-Wilhelm Radue, Ludwig Kappos, Till Sprenger

University Hospital Basel, Switzerland

Background. Previous studies that investigated motor learning in patients with multiple sclerosis (MS) showed improvement of upper limb function (Leocani et al., 2007), gait (Baram et al., 2006) and head control (Cattaneo et al., 2005) after training. Despite the crucial role of motor skill learning for everyday life and rehabilitation procedures, the underlying neural mechanisms have not been studied in detail.

Aim. We aim to use fMRI in order to investigate functional cerebral changes induced by short-term motor training in patients with relapsing-remitting MS (RRMS).

Methods. So far, we enrolled 10 patients (mean age 35±4.6 years; 5 women; total number at end of study will be 20 patients) with RRMS and 14 controls (average 32±5.7 years; 8 women; without deficit in upper extremities). Each patient had a neurological examination (median EDSS: 2; range: 1-4,) and both groups underwent a neuropsychological assessment including 9-HPT, PASAT, visual memory span and n-back task. The MRI data were acquired on a 3T Prisma scanner (Siemens Medical, Germany) including high resolution 3D T1-weighted images, high resolution 3D FLAIR and EPI images (TR/TE=2500/30 ms, isotropic voxel size 3 mm, 202 volumes). The functional MRI (fMRI) paradigm consisted of a sequence learning task with two conditions: trained and random sequences. Specifically, subjects had to press keys on a 4-button keyboard as quickly and accurately as possible following a cue presented on a screen. Subjects performed fifteen training blocks and fifteen random finger-sequence blocks with the right hand. In each block a 10-item finger sequence was presented. fMRI data pre-processing and group comparisons were performed using FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). Results were corrected for multiple comparisons using a cluster thresholding approach.

Results. Patients with RRMS showed a reduced performance when learning the sequence task compared to healthy subjects (mean reaction time in the last trained sequence: 416 ms in controls vs 477 ms in patients; $p<0.05$). In the fMRI analysis patients had a reduced BOLD response (indicating reduced brain activation) in the middle frontal cortex (BA 10), precentral gyrus (BA6), and intra-parietal sulcus compared to healthy subjects in the training condition compared to the random sequence condition. No differences were observed in the outcomes of the neuropsychological assessment between MS patients and healthy subjects.

Conclusion. These preliminary results indicate that a reduced motor learning performance in MS patients goes along with reduced activity of the dorsal attentional network (fronto-parietal brain regions) during the training condition.

Acknowledgement. This study is being supported by the Swiss MS Society.

Role of CD8+ T cell and infection in an animal model of multiple sclerosis

Nicolas Page[1], Karin Steinbach[1], Dietmar Zehn[2], Daniel Pinschewer[3], Doron Merkler[1,4]

[1] *Department of Pathology and Immunology, CMU-University of Geneva*

[2] *Swiss Vaccine Research Institute and Division of Immunology and Allergy, Department of Medicine, Lausanne University Hospital*

[3] *Department of Biomedicine, University of Basel*

[4] *Division of clinical pathology, University Hospital Geneva*

Multiple sclerosis (MS) is the most common inflammatory demyelinating disease of the central nervous system (CNS) causing disability in young adults. Epidemiological observations suggest that microbial infections have the capacity to precipitate MS in genetically predisposed individuals, but unifying characteristics of such pathogens remain elusive. Furthermore, recent evidences suggest that $CD8^+$ cytotoxic T lymphocytes (CTLs) may be crucially involved in MS pathogenesis. However, most MS animal models are mainly driven by encephalitogenic $CD4^+$ T helper cells. Recently a transgenic mouse model expressing ovalbumin (OVA) as a neo-"self" antigen in oligodendrocytes was described (ODC-OVA mice). We took advantage of this new animal model, to monitor the microbial capacity of two recombinant pathogens expressing OVA (Listeria-OVA and LCMV-OVA) to elicit an encephalitogenic CTLs response.

We found that the expansion of adoptively transferred OVA-specific $CD8^+$ T (OT-1) was similar upon peripheral infection with either Listeria-OVA or LCMV-OVA, respectively. Strikingly, peripheral infection with LCMV-OVA but not with Listeria-OVA induced EAE-like symptoms that were associated with locomotor impairments in ODC-OVA mice. Histopathological analysis revealed inflammatory demyelinating lesions that were predominated by CTL infiltrates in the brain and spinal cord upon pathogen challenge. We further assessed

the clonal differentiation phenotype (effector/memory), cytokine production (IL-2, TNF-α, IFN-γ, IL-10), degranulation activity (CD107a) and cytotoxic activity of LCMV-OVA or Listeria-OVA primed OT-1. To identify a gene expression signature that may characterize encephalitogenic OT-1 cells we performed a transcriptome analysis of FACS-sorted brain infiltrating OT-1 cells. We found that although the core transcriptional signatures were regulated similarly after Listeria-OVA or LCMV-OVA infection, some genes were differential regulated in diseased versus non-diseased animals. We are currently validating those identified candidate genes in functional readouts. Our study may thus provide new insights into the underlying mechanisms that govern functional and transcriptional regulation of an encephalitogenic CTLs.

The novel FTY720 derivative ST-968 improves symptoms of active experimental autoimmune-induced encephalomyelitis in mice independent of SphK2 by directly enhancing the blood-brain barrier function.

Faik Imeri[1], Aleksandra Zivkovic[3], Holger Stark[3], Ruth Lyck[4], Britta Engelhardt[4], Josef Pfeilschifter[2], and Andrea Huwiler[1,2],*

[1] *Institute ofPharmacology, University of Bern*

[2] *Pharmazentrum Frankfurt/ZAFES, University Hospital, Goethe University Frankfurt am Main, Germany.*

[3] *Institute of Pharmaceutical Chemistry, Goethe University Frankfurt am Main, Germany.*

[4] *Theodor-Kocher Institute, University of Bern.*

The immunomodulatory drug FTY720 (fingolimod) is presently approved for the treatment of relapsing-remitting multiple sclerosis. It acts as a functional antagonist on the sphingosine-1-phosphate (S1P) receptor on T lymphocytes and thereby induces their homing to secondary lymphoid tissue. In this study, we have investigated the role of sphingosine kinase 2 (SK-2) in experimental autoimmune-induced encephalomyelitis (EAE) in mice. We show that mice deficient in SK-2 have significantly reduces clinical symptoms of EAE. Furthermore, SK-2 was found to be essential for FTY720 to exert its protective effect in EAE since SK-2 deficient mice were no longer protected. However, the previously reported FTY720

oxazolo-oxazole derivative, ST-968, which was characterized as a non-pro-drug substance with a similar S1P1 functional antagonistic property, was still fully active in SK-2 deficient mice and reduced clinical symptoms significantly. Spinal cord sections of SK-2 deficient EAE mice revealed that not only infiltrating immune cells were reduced but also the blood-brain-barrier (BBB) leakage was reduced compared to wildtype EAE mice. mRNA expressional studies of isolated brain and spinal tissue showed that the mRNA expressions of ICAM-1 and VCAM-1, as well as of matrix metalloproteinase-9 were reduced in SK-2 deficient mice, whereas PECAM-1 and the tissue inhibitor of metalloproteinase TIMP-1 were upregulated.

In summary, we showed here that loss of SK-2 mediates a protection from EAE by a dual mechanism, on the one hand by reducing immune cell infiltration and on the other hand by directly enhancing the barrier function of the BBB. FTY720 proved to be dependent on the presence of SK-2, whereas the novel non-pro-drug substance ST-968 acted protective in a SK-2 independent way.

Home-based training to improve manual dexterity in patients with multiple sclerosis: a randomized controlled trial.

Christian P. Kamm[1], Heinrich P. Mattle[1], René M. Müri[2], Mirjam R. Heldner[1], Verena Blatter[1], Sandrine Bartlome[2], Judith Lüthy[2], Debora Imboden[2], Giovanna Pedrazzini[1], Stephan Bohlhalter[4], Roger Hilfiker[3], Tim Vanbellingen[1,4]

[1] *Department of Neurology, Inselspital, Bern University Hospital, and University of Bern*
[2] *Division of Cognitive and Restorative Neurology, Department of Neurology, Inselspital, Bern University Hospital, and University of Bern*
[3]*HES-SO Valais-Wallis, School of Health Sciences, University of Applied Sciences Western Switzerland Valais, Sion,*
[4]*Neurology and Neurorehabilitation Center, Luzerner Kantonsspital*

Background: Impaired manual dexterity is frequent and disabling in patients with multiple sclerosis (MS) affecting activities of daily living (ADL) and quality of life.

Objective: To evaluate the effectiveness of a standardized, home-based training program to improve manual dexterity and dexterity related ADL in MS patients.

Methods: Randomized, rater-blinded controlled trial. Thirty-nine MS patients acknowledging impaired manual dexterity and having a pathological Coin Rotation Task (CRT), Nine Hole Peg Test (9HPT) or both were randomized 1:1 into two standardized training programs, the dexterity training program and the theraband training program. Patients trained five days per week in both programs over a period of 4 weeks. Primary outcome measures performed at baseline and after 4 weeks were the CRT, 9HPT and a dexterous related ADL questionnaire. Secondary outcome measures were the Chedoke Arm and Hand Activity Inventory (CAHAI-8) and the JAMAR test.

Results: The dexterity training program resulted in significant improvements in almost all outcome measures at study end compared to baseline. The theraband training group resulted in considerable and partly significant improvements as well.

Conclusion: The home-based dexterity training program significantly improved manual dexterity and dexterity related ADL in moderately disabled MS patients.

Low threshold of activation of human memory TH17 cells dependent on miR-181a

Federico Mele, Camilla Basso, Cristina Leoni, Simone Becattini, Dominik Aschenbrenner, Daniela Latorre, Antonio Lanzavecchia, Federica Sallusto and Silvia Monticelli

Institute for Research in Biomedicine, Bellinzona, Switzerland

CD4 T helper (Th) lymphocytes have a central role in orchestrating immune responses to invading pathogens. Th17 cells are characterized by the ability to produce IL-17 and IL-22, and are involved in epithelial and neutrophil-mediated immune responses against extracellular pathogens, such as bacteria and fungi. However, inflammatory Th17 cells are also known to play prominent roles in the pathogenesis of autoimmune diseases such as rheumatoid arthritis and multiple sclerosis. Using a variety of biochemical and cellular approaches, we identified a novel regulatory mechanism originating from T cell receptor (TCR) signaling, and predominantly functioning in Th17 cells, as compared to other Th subsets. This mecha-

nism involves sustained ERK phosphorylation, induction of negative regulators of transcription as well as miRNAs. This molecular network modulated Th17 threshold of activation and proliferation, and regulated the downmodulation of IL-17 expression in recently activated human Th17 cells. Importantly, if such a mechanism is dysregulated, Th17 cell-induced autoimmunity may occur or be exacerbated. A better characterization of these aspects of Th17 cell biology and IL-17 production is essential for the development of novel therapeutic agents for the treatment inflammatory and autoimmune diseases.

Molecular mechanisms and cellular pathways involved in the migration of CD8+ T cell subsets across the BBB in vitro and in vivo.

Henriette Rudolph[1, 2], Armelle Klopstein[1], Claudia Blatti[1], Britta Engelhardt[1].

[1] *Theodor Kocher Institute, University of Bern*

[2]*Pediatric Infectious Diseases, University Children's Hospital Mannheim, Heidelberg University, Mannheim, Germany*

Multiple sclerosis (MS) is the most common inflammatory disease of the central nervous system (CNS). It is characterized by an important infiltration of circulating immune cells into the CNS parenchyma, which results in neuroinflammation and demyelination.

For decades, the $CD4^+$ T lymphocytes have been thought to be the main effector cells in MS, but the potential importance of $CD8^+$ T lymphocytes in this disease has recently emerged. Indeed, analysis of T cell infiltrates in inflammatory lesions in post-mortem MS brains revealed that $CD8^+$ T cells are more numerous than $CD4^+$ T cells. However, the anatomical routes and the molecular mechanisms used by $CD8^+$ T cells to traffic to the CNS remain poorly investigated. Interestingly, relapsing-remitting MS patients treated with Natalizumab (a humanized anti- α4-integrin antibody) has proven efficiency but is associated with an increased risk of developing progressive multifocal leukoencephalopathy (PML), an often fatal disease of the CNS caused by JC virus infection. As $CD8^+$ T cells are in charge of controlling virus infections, de-

velopment of PML is thought to be due to Natalizumab-mediated inhibition of CNS entry and thus immunosurveillance by virus-specific $CD8^+$ T cells.

To determine if $CD8^+$ T cells use similar or distinct mechanisms to cross the blood brain barrier (BBB), we have started to directly compare the dynamic behavior of $CD8^+$ T cells versus $CD4^+$ T cells with the inflamed primary mouse brain microvascular endothelial cells (pMBMECs), as an *in vitro* BBB model, under physiological flow. We observed that $CD8^+$ T cells are significantly more efficient than $CD4^+$ T cells in arresting on pMBMECs and this was less dependent of ICAM-1 and ICAM-2. Moreover, we observed that most of the $CD8^+$ T cells remained stationary or crawled for a short distance before crossing the BBB compared to $CD4^+$ T cells that crawled for longer distances before diapedesis. In the absence of endothelial ICAM-1 and ICAM-2 $CD4^+$ T cells failed to crawl and diapedese, whereas 25% of arrested $CD8^+$ T cells were able to cross the pMBMECs. Our data highlight the different mechanisms used by $CD8^+$ T cells versus $CD4^+$ T cells in crossing the inflamed BBB *in vitro* and might offer new target for efficient blockade of CNS recruitment of destructive immune cells. The *in vivo* relevance of these findings needs to be further clarified.

Anti-MOG immune response characterizes a subgroup of AQP4-seronegative patients with a NMO phenotype

A.K. Proebstel[1,2], G. Rudolf[3], N. Sanderson[2], K. Dornmair[4], N. Collongues[3], J.B. Chanson[3], L. Kappos[1,2], J. De Sêze[3]*, T. Derfuss[1,2]*. These authors contributed equally.

[1] *Department of Neurology, University Hospital Basel*

[2] *Department of Biomedicine, University of Basel*

[3] *Department of Neurology, Hôpital de Hautepierre, Hôpitaux Universitaires de Strasbourg*

[4] *Institute of Clinical Neuroimmunology, University Hospital Grosshadern, Munich*

Background: Neuromyelitis optica (NMO) is a clinically defined autoimmune inflammatory disorder of the central nervous system. Antibodies against aquaporin-4 (AQP4) are present in

50-90% of the patients. Recent evidence hints at the presence of anti-myelin oligodendrocyte glycoprotein (MOG) antibodies in some AQP4-seronegative patients. However, the frequency as well as the pathogenic, diagnostic and prognostic relevance of these anti-MOG antibodies remains unclear.

Objective: To determine the frequency of antibodies against native MOG in AQP4-seronegative and -seropositive NMO and multiple sclerosis (MS) patients and to correlate anti-MOG antibody findings with clinical and MRI characteristics. Moreover, we also aimed at investigating the cellular immune response against MOG.

Methods: We analyzed the presence of anti-MOG immunoglobulins (IgG) in a total of 101 sera from patients with NMO (n=53) and MS (n=48) in a blinded fashion. Anti-MOG IgG reactivity was determined by the ratio of the geometric mean channel fluorescence (GMCF) of the transfected and the untransfected cell lines in flow cytometry. The cut-off was calculated to be 1.45 (mean GMCF ratio plus two standard deviations of a control group measured in parallel). Immunofluorescent stainings with anti-MOG IgG and analysis of the cellular immune response against MOG are carried out in parallel.

Results: 6 out of 53 patients with clinically definite NMO tested positive for IgG against native conformational MOG. All of them are anti-AQP4 seronegative. The anti-MOG IgG positive patients differed from anti-AQP4 IgG positive patients in terms of epidemiological (MOG vs. AQP4 reactivity: female 67 vs. 74%; mean age 50 vs. 45 years), clinical (first presentation with both myelitis and optic neuritis 50 vs. 16%, presence of OCBs 60 vs. 16%, median EDSS at sample date/follow-up 3.8/2.8 vs. 3.0/3.0) and MRI features (brain lesions 50 vs. 16%, longitudinally extensive transverse myelitis 83 vs. 87%). None of the 48 patients with MS had anti-MOG IgG.

Conclusion: Presence of anti-MOG IgG characterizes a subgroup (6/22, 27.3%) of AQP4-seronegative patients with NMO, but is absent in AQP4-seropositive NMO and MS patients. Anti-MOG IgG positive patients seem to have a distinct clinical phenotype compared to anti-AQP4 IgG positive patients with a more severe initial presentation but more favorable outcome.

Funding: This study was partly funded by the Swiss Multiple Sclerosis Society.

Disclosures: A.K. Proebstel is supported by a research fellowship from Genzyme. G. Rudolf has nothing to disclose. N. Sanderson has nothing to disclose. K. Dornmair has nothing to disclose. N. Collongues has nothing to disclose. J.B. Chanson has nothing to disclose. L.

Kappos received institutional research support from Acorda, Actelion, Allozyne, BaroFold, Bayer HealthCare, Bayer Schering, Bayhill, Biogen Idec, Boehringer Ingelheim, Elan, Genmab, Glenmark, GlaxoSmithKline, Merck Serono, MediciNova, Novartis, sanofi-aventis, Santhera, Shire, Roche, Teva, UCB, Wyeth, Swiss MS Society, Swiss National Research Foundation, European Union, Gianni Rubatto Foundation, the Novartis and Roche Research Foundation. J. De Sêze has nothing to disclose. T. Derfuss serves on scientific advisory boards for Novartis Pharma, GeNeuro, Octaparma, Merck Serono, Biogen Idec, Genzyme, Mitsubishi Pharma, TEVA Pharma, and Bayer Schering Pharma; has received funding for travel and/or speaker honoraria from Biogen Idec, Novartis, Merck Serono, and Bayer Schering Pharma; and receives research support from the Swiss National Fund, Novartis Pharma, the European Union, and the Swiss MS Society.

Oxysterols regulate encephalitogenic CD4+ T cell trafficking during experimental autoimmune encephalomyelitis

Chalmin F[1,2], Rochemont V[1,2], Lippens C[2], Clottu A[2], Sailer AW[3], Merkler D[2], Hugues S[2], Pot C[1,2]

[1] *Division of Neurology, Department of Clinical Neurosciences, Geneva University Hospitals*

[2] *Department of Pathology and Immunology, University of Geneva*

[3] *Developmental and Molecular Pathways, Novartis Institutes for BioMedical Research, Basel, Switzerland.*

Perturbation of steroids pathways is linked to inflammation and chronic diseases, however the underlying mechanism remains unclear. Oxysterols, oxidised forms of cholesterol, have recently been shown to contribute to the immune response and have been proposed as candidate biomarkers for neurological diseases such as Multiple sclerosis (MS). The enzyme cholesterol 25 hydroxylase (ch25h) is the rate limiting step to synthetize both 25-hydroxycholesterol (25-OHC) and 7α,25-dihydroxycholesterol (7α25-OHC) from cholesterol. In addition to their basic metabolic properties for bile synthesis and sterol transportation, 25-OHC controls viral infection and 7α25-OHC guides B cells and macrophages within

germinal centers. However how oxysterols modulate adaptive immunity is largely unknown and their role in autoimmunity has not been evaluated.

We assessed the role of Ch25h in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis and investigated the underlying mechanisms. We report that deletion of ch25h attenuated EAE disease course by limiting pathogenic T lymphocytes trafficking to the central nervous system (CNS). Mechanistically, we show a critical involvement for oxysterols in recruiting leukocytes into inflamed tissues and show that 7α,25-OHC preferentially promotes the migration of subset of inflammatory T lymphocytes both *in vitro* and *in vivo*. Collectively, our results revealed a critical involvement for oxysterols in the development of EAE and on T lymphocytes trafficking. Overall, not only these findings highlight a pro-inflammatory role of 7α,25-OHC during EAE, but identify oxysterols as potential therapeutic targets to inhibit development of autoimmunity.

Inhibition of T cell mediated neuroinflammation by Btn2a2, a novel immunomodulatory molecule co-regulated with MHC class II genes

Kerstin Sarter, Elisa Leimgruber, Fernanda Do Valle Duraes, Leslie Guéry, Florian Gobet, Stéphanie Hugues and Walter Reith

Department of Pathology and Immunology, Faculty of Medicine, University of Geneva

Butyrophilin (BTN) and butyrophilin-like (BTNL) proteins have recently gained importance in immunology due to the observations that their members can alter T cell responsiveness (BTNL2), drive intra-thymic differentiation of Vγ5Vδ1 T cells (Skint1), mediate activation of Vγ9Vδ2 T cells (BTN3A1) and bind to DC-SIGN (BTN2A1). Furthermore, genetic polymorphisms in the *BTNL2* gene have been associated with predisposition to inflammatory human diseases, including sarcoidosis, ulcerative colitis, rheumatoid arthritis, systemic lupus erythematosus, and type I diabetes. These findings suggest that improving our understanding of the roles that BTN molecules play in the modulation of T cell responses *in vivo* could lead to new applications in the prevention of excessive immune responses responsible for autoimmune conditions and inflammatory disorders.

BTN molecules constitute a family of transmembrane proteins belonging to the immunoglobulin domain superfamily. They exhibit strong homology to the B7 family of co-stimulatory and immunomodulatory molecules. There are 7 members of the BTN family in humans, whereas there are only two (Btn1a1 and Btn2a2) in mice. In humans and mice, *BTN* genes are clustered in the extended MHC class I region on chromosome 6 and chromosome 13, respectively. The functions of BTN proteins remain largely unknown.

We recently discovered that expression of the human *BTN2A2* and mouse *Btn2a2* genes is co-regulated with MHC class II (*MHCII*) genes. Their expression in antigen presenting cells (APCs) is controlled by CIITA, the master regulator of *MHCII* genes, and RFX, a key component of the enhanceosome complex that assembles on an enhancer characteristic of *MHCII* promoters. Furthermore, similarly to *MHCII* genes, *Btn2a2* expression is activated by IFNγ-mediated induction of CIITA in fibroblasts and endothelial cells. These findings suggest that Btn2a2 might play a role in antigen-presentation and/or the modulation of T cell mediated immune responses. This hypothesis would be consistent with the observations that a) Btn molecules are related to B7 molecules, b) Btn2a2 expression has been documented on APCs, including $CD19^+$ B cells, $CD11c^+$ splenic DCs and $CD11b^+Ly6G^{low}$ peritoneal macrophages, c) Btn2a2 can bind to and inhibit the proliferation of activated $CD4^+$ and $CD8^+$ T cells *in vitro,* d) Btn2a2 expression is induced by IFNγ in fibroblasts and endothelial cells, and e) the *Btn2a2* gene is regulated by the MHCII-specific transcription machinery.

To investigate the function of Btn2a2 *in vivo* we generated *Btn2a2* knockout (*Btn2a2*$^{-/-}$) mice. As Btn2a2 was proposed to inhibit T cell responses we reasoned that a T cell-mediated autoimmune disease such as EAE could be aggravated in *BTn2a2*$^{-/-}$ mice. We found out that MOG-induced EAE was indeed exacerbated in *Btn2a2*$^{-/-}$ mice. *Btn2a2*$^{-/-}$ mice exhibited an accelerated disease onset, higher cumulative and maximum disease scores, and a higher disease incidence compared to WT mice. At disease onset (8 days after induction) percentages of IFNγ-producing CD4+ T cells in draining lymph nodes and the spinal cord were significantly increased in *Btn2a2*$^{-/-}$ mice compared to WT controls. At peak disease (day 15 after induction) *Btn2a2*$^{-/-}$ mice also exhibited significantly increased numbers of infiltrating $CD4^+$ T cells and a lower percentage of $CD4^+Foxp3^+$ Tregs in the spinal cord, as well as higher percentages of IL17-producing $CD4^+$ T cells in the spinal cord and draining lymph nodes. EAE experiments performed with bone marrow chimeras (*Btn2a2*$^{-/-}$ into wt and wt into *Btn2a2*$^{-/-}$) demonstrated that exacerbated disease is due to the loss of Btn2a2 expression by

cells of the hematopoietic compartment. Lastly, preliminary data using OTII transfer experiments suggest that Btn2a2 expression in APCs modulates T cell responses *in vivo*.

Taken together, these *in vivo* results demonstrate that Btn2a2 is a novel negative regulator of T cell responses and that it exerts a marked inhibitory effect on the development of T cell mediated neuroinflammation.

Transfer of plasmacytoid Dendritic Cells lead to therapeutic abrogation of EAE through endogenous plasmacytoid DC recruitment and modulation in the CNS

Duraes, FV*; Lippens C*; Steinbach K; Merkler D; Reith W; Hugues S.

Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Switzerland
** These authors equally contributed to this work.*

Plasmacytoid dendritic cells (pDCs) constitute a unique subtype of dendritic cells (DCs) that is well-known to participate in innate immune responses due to their elevated production of type-1 interferons upon activation by microbial stimuli. pDCs play also important roles in the pathogenesis of several diseases. However, their contribution to Multiple Sclerosis (MS) remains very controversial, with pDCs exerting either pro-pathogenic or tolerogenic roles. Using a mouse model of MS, EAE, we have previously demonstrated that adoptive transfer of pDCs prior to EAE induction conferred significant protection against the disease. In this model, MHCII-mediated antigen presentation by pDCs promotes the expansion of regulatory T cells (Treg) capable of suppressing encephalitogenic T cells. In order to explore a potential therapeutic role for pDCs in EAE, we adoptively transferred pDCs into C57BL/6 mice after EAE onset, at the peak disease phase. pDC transfer induced significant inhibition of EAE clinical symptoms. Surprisingly, this protection was observed using both MHCII sufficient and deficient pDCs, suggesting an antigen-presentation independent function of injected pDC in mediating EAE protection. Correlating with disease inhibition, inflammatory foci, MHCII expression by microglia cells and T effector responses were impaired in the CNS of mice transferred with pDCs. By transferring GFP^+ pDCs we were able to visualize these cells by confocal microscopy and flow cytometry in the CNS as well as in peripheral lym-

phoid organs. Moreover, pDC generation in the BM was increased upon pDC transfer and correlated with a massive and selective recruitment of endogenous pDCs to the CNS, via a chemerin-dependent mechanism. In addition, pDC activation state was locally down-modulated in the CNS of pDC-transferred mice. The cellular and molecular mechanisms involved in pDC-mediated EAE inhibition are currently under investigation. Given that MS patients display altered pDC numbers and functions, unraveling the mechanisms underlying the protective role of pDC transfer could bring to light an important therapeutic role for pDCs in MS.

B) Poster Presentations

Detailed in vitro analysis of the molecular and cellular pathway of T cell extravasation across the highly specialized BBB endothelium

Michael Abadier[1], Neda Haghayegh Jahromi[1], Urban Deutsch, Britta Engelhardt and Ruth Lyck

Theodor Kocher Institute, University of Bern

[1] Graduate School for Cellular and Biomedical Sciences, University of Bern

In multiple sclerosis (MS) or its animal model experimental autoimmune encephalomyelitis (EAE), extravasation of autoagressive T cells into the CNS is a crucial step in disease progression. Within the CNS the microvasculature is highly specialized to build up the tight blood-brain barrier (BBB). Extravasation of CD4+ effector/memory T (TEM) cells across the inflamed BBB is a tightly regulated multi-step process initiated by T cell rolling and capturing on the luminal surface of the endothelial cells, followed by T cell shear resistant arrest, polarization, crawling and, finally, diapedesis. Each of these steps is tightly regulated through defined molecular interactions between the T cells and components of the BBB. Therapeutical targeting of immune cell trafficking into the CNS with the monoclonal antibody Natalizumab (Tysabri®) has proven beneficial for treatment of MS patients. However, Natalizumab can cause unwanted side effects like the mobilization of CD34+ hematopoietic progenitor cells and in rare cases the fatal re-activation of JC virus leading to progressive multifocal leukoencephalopathy (PML). Our research is designed to gain a better understanding of the differential roles of endothelial cell surface molecules for the individual steps of CD4+ TEM cell extravasation.

Using live cell imaging of CD4+ TEM cell interaction with TNF-α stimulated primary mouse brain microvascular endothelial cells (pMBMECs) under flow conditions as a most physiological *in vitro* model for the inflamed blood-brain barrier (BBB), we have previously demonstrated the essential roles of endothelial ICAM-1 and VCAM-1 in mediating shear resistant arrest of encephalitogenic CD4+ TEM cells on the inflamed BBB, whereas endothelial ICAM-1 and ICAM-2 but not VCAM-1 supported sustained shear resistant adhesion and T cell crawling against flow to sites permissive for diapedesis. Endothelial ALCAM, howev-

er, is not required for the dynamic interaction of CD4+ TEM cells with the inflamed BBB or for the development of EAE. Most recently, we investigated the influence of differential cell surface levels of endothelial ICAM-1 in determining the cellular route of CD4+ TEM cell diapedesis which can be via the endothelial junctions (paracellular) or via a pore across the endothelial cytoplasma (transcellular). Inflammatory conditions inducing high cell surface expression levels of endothelial ICAM-1 promoted rapid initiation of transcellular diapedesis of CD4+ TEM cells across the BBB, while intermediate levels of endothelial ICAM-1 favored paracellular CD4+ TEM cell diapedesis. Importantly, the route of CD4+ TEM cell diapedesis across the BBB was independent of loss of BBB barrier properties. Unexpectedly, in the absence of endothelial ICAM-1 and ICAM-2 when only few CD4+ TEM cells adhere and diapedese without any crawling, most diapedesis events were via the transcellular pathway. *In vivo*, ICAM-1null//ICAM-2-/- C57BL/6J mice lacking all isoforms of ICAM-1 and ICAM-2 developed ameliorated EAE. These findings suggest that targeting ICAM-1 and ICAM-2 in MS patients cannot completely abrogate CNS invasion by encephalitogenic CD4+ TEM cells.

Short-term immune adaptations to different endurance training protocols (aquatic versus overland) in MS and their relation to health-related quality of life, fatigue and cardiorespiratory fitness after three weeks randomized controlled trial

J. Bansi[1], W. Bloch[2], U. Gamper[1], S. Riedel[3], J. Kesselring[1]

[1] *Kliniken – Valens, Rehabilitationsklinik Valens*

[2] *German Sport University Cologne, Institute of Cardiology and Sports Medicine, Cologne, Germany*

[3] *arignos, Dresden, Germany*

Background. The influences of exercise on cytokine response, health related quality of life (HR-QOL) and fatigue are important aspects of MS rehabilitation. Physical exercises performed within these programs are often practised in water but the effects of immersion have not been investigated.

Objectives. To investigate the influences of short-term immune adaptations on HR-QOL, fatigue and cardiorespiratory fitness during three weeks endurance training conducted on a cycle-ergometer or an aquatic-bike.

Methods. Randomized controlled clinical trial in 60 MS patients. HR-QOL, fatigue, cardiorespiratory fitness and short-term immune changes (serum concentrations in response to cardiopulmonary exercise test) of brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), Interleukin-6 and the soluble receptor of IL-6 (sIL-6R) were determined at begin and end of three weeks training intervention. Subjects performed daily 30 Min. training at 60% of their VO_2peak.

Results. SF-36 total (p=0.031), physical (p=0.004) and mental health (p = 0.057) scores show time effects within both groups. Between group effects were shown for FSMC total (p=0.040) and motor function score (p=0.041). MFIS physical fatigue showed time effects (p=0.008) inside both groups. Linear regression models showed relationships between HR-QOL, fatigue, cardiorespiratory fitness and the short-term immune changes after the intervention.

Conclusion. This study indicates beneficial effects of endurance training independent the training setting. Short-term immune adaptations have the potential to influence HR-QOL, fatigue and cardiorespiratory fitness in persons with MS (PwMS). The specific immune responses of immersion to exercise need further clarification.

Extra-lymphatic CCR7-ligand expression controls virus-induced CNS inflammation

Jovana Cupovic[1], Cristina Gil-Cruz[1], Lucas Onder[1], Elke Weiler[2], Ingo Bachmann[2], Sonja Firner-Caviezel[1], Christian Perez-Shibayama[1] and Burkhard Ludewig[1]

[1] *Institute for Immunobiology, Kantonal Hospital St. Gallen*

[2] *Institute of Anatomy, University of Leipzig, Germany*

The constitutive chemokines CCL19 and CCL21, i.e. the ligands of chemokine receptor 7 (CCR7), are critical regulators of immune homeostasis and activation within secondary lymphoid organs. Importantly these chemokines are also expressed in the CNS and their expres-

sion is significantly elevated in patients suffering from multiple sclerosis. However, it has remained elusive whether and how these chemokines impinge on virus-driven inflammatory processes in the CNS that precipitate demyelination. Using Ccl19-Cre reporter mice facilitating in vivo tracking of CCL19-producing cells, immunohistochemistry and RT-PCR analysis, we assessed to which extent CCR7 ligands produced in the CNS contribute to the control of coronavirus-mediated CNS inflammation. We found that CCL19 was produced predominantly by endothelial cells of brain microvessels, astrocytes and stromal cells adjacent to the axonal trajectories of infected neurons. Moreover, we identified podoplanin-expressing endothelial cells of blood-brain-barrier microvessels as main producers of CCL21. To assess whether the disruption of the CCR7-CCL19/CCL21 axis in the CNS impacts on virus-induced CNS inflammation, we utilized mice lacking either CCR7 on all cells or CCR7-ligands selectively within secondary lymphoid organs (paucity of lymph node T cell (plt/plt) mice). CCR7-deficient mice rapidly lost weight and were terminally ill at day 10 post infection. In strong contrast, plt/plt mice that have preserved expression of the Ccl21-Lec isoform in the CNS, completely recovered from the infection. Moreover, antiviral T cell responses in CCR7-deficient mice were more severely affected compared to plt/plt mice resulting in impaired virus control. Importantly, competitive adoptive transfer of CCR7-proficient and CCR7-deficient, virus-specific $CD8^+$ T cells resulted in preferential recruitment of CCR7-expressing cells to the brains of plt/plt animals further showing that CNS-restricted expression of CCR7 crucially regulates cell recruitment to the brain. Further along the same line, adoptive transfer of CCR7 proficient virus-specific cells rescued CCR7-deficient mice from lethal CNS inflammation. Taken together, these data indicate that expression of CCR7 ligands in the CNS provides a crucial advantage for the host during neurotropic viral infection.

Functional connectivity of resting state EEG in MS patients: follow-up over two years

M. Hardmeier, F. Hatz, I.K. Penner, Y. Naegelin, H. Bousleiman, C. Schindler, L. Kappos, P. Fuhr

University Hospital Basel

Background: Functional connectivity is a promising tool to characterize and analyze alterations of oscillating brain activity. In MS the significance of such alterations is not well known.

Objective: To explore the dynamics of functional connectivity in MS over time.

Methods: 55 RRMS-patients (76% female; median age: 38.4 yrs, interquartile range (IQR): 33.2-44.5 yrs; median EDSS: 2.0; IQR: 1.5-3.0) and 35 normal controls (80% female; median age: 38.4 yrs, IQR: 30.4-43.2 yrs) received a 256 channel EEG at baseline and at years 1 and 2, and were assessed by the EDSS, the symbol digit modalities test (SDMT) and the fatigue scale for motor and cognitive functions (FSMC, Penner et al. 2009). Functional connectivity between 22 regions was determined by the weighted phase lag index (wPLI, Vinck et al. 2011) using TAPEEG (Hatz et al., 2014). The connectomes and the average connectivity of each region were calculated in four frequency bands (theta, lower and upper alpha, beta) and compared between groups and over time by permutation t-tests and ANOVA.

Result: Compared with normal controls, patients had reduced cognitive processing speed (SDMT: 56.2 vs. 63.8, $p<0.01$) and higher fatigue scores (FMSC: 51.9 vs. 33.2, $p<0.001$), but were stable over time regarding EDSS, SDMT, and FSMC (all $p>0.1$). Connectivity in the beta-band was reduced in MS patients, most significantly over left temporo-parieto-occipital regions ($p<0.05$ corrected) at baseline, and remained significantly different in years 1 and 2 (year 1: $p<0.05$ uncorrected, year 2: $p<0.05$ corrected). The upper alpha band showed a less consistent pattern of slightly reduced connectivity ($p<0.05$ uncorrected) involving also left temporo-parieto-occipital regions. Within MS-patients, connectivity did not change significantly over time in all frequency bands, and no differences to healthy controls were apparent in the lower alpha and theta-band.

Conclusions: Reduced connectivity over the left temporo-parieto-occipital regions in the beta-band discriminates between groups of MS patients and healthy controls, but does not change over time. However, on average, patients neither experience a change in clinical status, cognitive function nor fatigue during the observation period. Thus it remains unclear whether reduced beta-connectivity represents a marker of state or of trait.

Disclosure: The study has been financially supported by grants of the Swiss National Science Foundation (grants # 33CM30-124115/1; # 33CM30-140338; # 326030-128775/1) as well as the Swiss Multiple Sclerosis Society.

Homeostatic T cell proliferation in multiple sclerosis and its functional involvement in disease pathogenesis

Ivan Jelcic[1], Faiez Al Nimer[1], Ilijas Jelcic[1,2], Raquel Planas[1], Mireia Sospedra[1], Roland Martin[1,2]

[1] *Neuroimmunology and Multiple Sclerosis Research Section, Department of Neurology, University Hospital Zurich*

[2] *Department of Neurology, University Hospital Zurich*

Multiple sclerosis (MS) is a T cell-mediated demyelinating autoimmune disease of the central nervous system (CNS) causing neurological deficits with substantial disability at an early age. Both environmental factors and a complex genetic trait contribute to MS risk. The importance of the DR15 haplotype as genetic risk factor for MS is known since 1973 and was the first HLA-class II association that has been described for an autoimmune disease. The two HLA-class II alleles expressed in the HLA-DR15 haplotype (DRB1*15:01, DRB5*01:01), contribute far more to genetic risk than any other gene. However, it is until now poorly understood how these HLA-class II-molecules functionally contribute to MS pathogenesis. Among several possibilities it is most likely that peptides presented by these two HLA class II molecules contribute both to shaping a potentially pathogenetic T cell repertoire by central tolerance mechanisms in the thymus and/or maintaining and expanding these T cells in the peripheral immune system.

Recent observations by our group demonstrate increased autologous proliferation of peripheral lymphocytes in MS, particularly in HLA-DR15-positive MS patients, which involves HLA-DR15-derived self-peptides as potential mediators of this phenomenon in MS. However, the components of enhanced autologous proliferation and its relevance in MS pathogenesis remain to be shown.

In the present study, we aimed to analyze: (i) which T cell subpopulations and antigen-presenting cells are involved in increased autologous proliferation, (ii) if selection of a restricted T cell repertoire emerges and (iii) whether autologous proliferating T cells recognize HLA-DR-derived self-peptides and have the propensity to cross-react with CNS autoantigens or foreign antigens.

We collected samples from healthy donors and therapy-naïve MS patients, which were grouped according to their HLA-DR15 status by HLA genotyping and analyzed for autolo-

gous proliferation by thymidine incorporation assay. Alterations of autologous proliferation in MS were also followed in the context of MS therapy using patients treated with fingolimod and natalizumab in cross-sectional studies. HLA-DR15-positive donors were screened for reactivity to the most stimulating HLA-DR15-derived peptides. The influence of antigen-presenting cells on autologous proliferation was analyzed by prior depletion of B cells and monocytes. To identify the relevant T cell subpopulations, we established a multicolor CFSE-based assay for flow cytometry that correlated with the results obtained by thymidine incorporation assay. The latter method allows the use of sorting the autologous proliferating cells from selected patients, subsequent expansion of T cell lines and T cell receptor sequencing. These T cell lines will be then used to study recognition to HLA-DR15-derived peptides, viral antigens as well as CNS antigens. Comparison of the T cell receptor repertoire of autologous proliferating cells with CNS infiltrates and reactivity of MS brain-derived T cell clones to HLA-DR15-derived peptides should provide the relevant link whether enhanced autologous proliferation contributes to MS pathogenesis. We expect with this study to gain novel important insights about the cell phenotype, the TCR repertoire and the spectrum of antigens involved in the altered autologous proliferation in MS patients and how the DR15 haplotype contributes to these phenomena.

Responsivity and MID of the German Multiple Sclerosis Questionnaire for Physiotherapists MSQPT®

N. A. van der Maas
Institut für Physiotherapieforschung, Biel.
Head of the Science Committee of the FPMS, Physioswiss.

Purpose: To improve the evaluation of the physiotherapeutic treatment of patients with MS the quality group of the Specialized Group for Physiotherapy in Multiple Sclerosis (FPMS) developed the Multiple Sclerosis Questionnaire for Physiotherapists (MSQPT®), a self-rating questionnaire which measures activities and participations in daily life as well as several bodily functions, attitudes and aspects of Quality of Life. The German MSQPT is a valid and reliable tool and is well accepted by physiotherapists. This study evaluates the responsivity of the German MSQPT and provides adequate MID estimates.

Relevance: Many MS-patients are in long-term physiotherapy treatment. The MSQPT is the

first appropriate patient-based device for physiotherapists to evaluate the treatment of MS patients on an individual base. For the use and interpretation of the MSQPT on an individual base, the ability to detect change (responsivity) and a threshold for change should be known.

Participants: Inclusion criteria: Patients with a diagnosed MS, who are in physiotherapeutic treatment because of MS, patients older than 18 years with native language German, EDSS score of less or equal 6.5. Exclusion criteria: Acute episode of MS, grave cognitive changes, bedfast patients, patients with distinct fatigue or able to perform less than 2 hours.

Methods: Study design: Longitudinal multicenter study. Setting: private practices, which were owned by members of the FPMS and physiotherapy departments of hospitals.

We used a combined anchor and distribution based approach to evaluate the responsivity of the total score, of the reliable and valid groups and the reliable and valid items of the MSQPT. Responsivity was evaluated using ES, SRM and Modified SRM. Global and detailed transition questions provided a range of anchor-based estimates for minimal change out of the perspective of the patient. Furthermore, detailed transition questions provided a range of estimates for minimal change out of the perspective of the therapist. The distribution-based estimates were provided by 0.5 and 0.33 SD, SEM and $MDC_{95.}$ To provide a base for threshold decision making, different cut-off values for a MID were tested for sensitivity and specificity against the transition questions results. The thresholds with best sensitivity and specificity were selected as estimates for MID on an individual level.

The measures used are valid and reliable with known MDC: Berg Balance Scale, 6 Minute Walking Test and 6 Meter Walking Test. The global transition questions were provided by item 1 of the MSQPT and item 1 of the HAQUAMS.

Results: The convenience sample of 60 patients has a plausible representativeness of the Swiss MS-population. Only the missing data of the patient transition questionnaire was too high (21%). All others tests had missing data between 0.13 and 5.4 %.

Responsivity: The ES for bettering and worsening range from small (minimum 0.46) to large (maximum 1.49). The SRM data show comparable results. The Modified SRMs for the population without change are very good and generally very low (0.00 to 0.14) except for item 4 (.27 to .60).

The anchor-based estimates were very low, comparable with the 0.33 resp. 0.5 SD and the SEM of the distribution based results. The MDC_{95} values were considerably higher.

Specificity and sensitivity testing of the ranges of anchor- and distribution-based estimates provided the following best MID estimates:

One Interval for Items 4, 8a, 8b and two (for better) resp. three (for worse) intervals for the balance group. 7 intervals for better and 11 for worse for the activity related group. A 17 interval change for the participation related group and finally an 18 (for worse) and 20 (for better) threshold for the total score.

Conclusion: The MSQPT is a responsive questionnaire with low estimates for MID. The estimates provided are based on a population with not many patients that were getting better. More research is needed with patients getting better to appreciate the value of the provided estimates.

Sequence Profiling the T-cell Receptor Repertoire in Twin Pairs Concordant and Discordant for Multiple Sclerosis

Brenda J. Reinhart and Roland Martin.
Neuroimmunology and MS Research, Department of Neurology, University Hospital Zurich

Genetic studies have repeatedly shown an association between MS and multiple genes involved in T cell function or development (IMSGC, 2013). The HLA-DR15 haplotype alone confers between 10-60% of the genetic risk to develop MS, suggesting a strong link between a patient's CD4+ T-cells and disease. However, monozygotic twins have only a 20-30% disease concordance rate for MS. Twins may be discordant for the disease because of differences in stochastic, environmental, or epigenetic factors. Environmental risk factors contributing to MS etiology include Epstein Barr virus (EBV) infection, low vitamin D3 levels and smoking, but the basis of their influence is unknown (Sospedra and Martin, 2005). One possibility is that environment and/or stochastic factors shape differences in twins' T-cell receptor (TCR) repertoires that create differences in the self-reactivity of their T cell populations. The TCR repertoire is generated by somatic recombination rather than germ line encoded, and the different foreign antigens to which individuals are exposed will result in expansion of different T cells in the periphery. Twins discordant for MS could simply have less similarity between their TCR repertoires than those concordant for disease.

Previous studies have reported conflicting results on the similarity of the TCR repertoire in concordant and discordant twins (Utz et al, 1993, Haegert et al, 2003, Somma et al, 2007). However, these studies either used spectratyping, which only examines CDR3 length bias within the TCR population of a particular V chain, or used qPCR to examine bias in the relative use of V chains without examining the CDR3 sequences. We are using next-generation sequencing to look at both relative V beta chain usage and detailed CDR3 sequences in the TCR repertoire of twins.

We have isolated memory CD4+ T cells from two sets of monozygotic twins, one concordant for MS and one discordant. Cells were either left unstimulated or stimulated for 36 hours with one of three foreign antigen mixes or a general anti-CD3/anti-CD28 stimulation to increase the levels of TCR mRNA in the cells responding to the stimulus. Total RNA was isolated from the cells, reverse transcribed into cDNA, and the TCRs are being amplified and sequenced by the Immunoseq method (Adaptive Biotechnologies). We plan to examine our data to determine if the twins concordant for MS respond more similarly to specific antigen stimuli than those discordant for MS, suggesting a role for the TCR repertoire in disease concordance.

Capture of Self Antigens from Plasma Membrane by B cells

Nicholas Sanderson, Maria Zimmermann and Tobias Derfuss

University Hospital Basel

We hypothesise that B cells contribute to multiple sclerosis via stimulation of autoreactive T cells following the adventitious capture of self antigens in the context of viral infection. The mechanism we envisage is restricted to membrane bound self antigens co-expressed with viral proteins whose extracellular domains are exposed to B cell surveillance. We have tested this hypothesis using haemagglutinin-specific B cells and transgenic T cells recognising a peptide derived from Myelin Oligodendrocyte Glycoprotein (MOG). B cells capture large amounts of their cognate antigen from non-professional antigen presenting cells, together with much smaller amounts of non-cognate "bystander" protein. Both are processed and presented at a sufficient level to cause proliferation of cognate T cells.

The absence of TNF exacerbates spontaneous neuroinflammation in MOG-specific TCR-transgenic (2D2) mice

Nora Schweizer[1], Cinzia Tiberi[2], Elisabeth Rushing[2], Hans Welzl[3], Mirjana Wojtal[1], and Tobias Suter[1]

[1] *Neuroimmunology and MS Research, University Hospital Zürich (USZ)*

[2] *Institute of Neuropathology, University Hospital Zürich*

[3] *Institute of Anatomy, University Zürich*

A small fraction of mice carrying the MOG-specific T cell receptor (TCR) 2D2 spontaneously develop autoimmune encephalomyelitis accompanied by demyelination of the central nervous system (CNS). These symptoms resemble the ones seen in human multiple sclerosis (MS). A greater fraction of 2D2 mice, however, develop peripheral neuropathy, the reason for which is thought to be the cross-reaction of the 2D2 TCR with a neurofilament M (NF-M) peptide.

Several cytokines play distinct roles during MS as well as experimental autoimmune encephalomyelitis (EAE), a commonly used animal model of MS. One of these cytokines, TNF, has been shown to exert pro-inflammatory as well as protective activities during neuroinflammation. However, the mechanisms and regulation of its dual role are incompletely understood.

In this study, we crossed TNF-deficient (TNFko) and 2D2 mice to further investigate the role of TNF in the context of spontaneous neuroinflammation. Beginning at the age of 3 months, approximately half of the 2D2-TNFko mice spontaneously developed clinical signs of neurological impairment. Interestingly, 2D2-TNFko mice rarely developed signs of typical EAE but suffered from a characteristic gait disturbance. Still, they showed increased infiltrates of CD4 and CD8 T cells in the CNS and activated T cells in the secondary lymphoid organs. Immunohistochemistry of the sciatic nerve of these mice revealed high expression of MHCII and the presence of lymphocytes and CD11b-positive myeloid cells. Toluidine blue staining pointed to severe peripheral neuropathy in the nerves of the hind limbs suggesting inflammation-mediated destruction of the nerves of the hind limbs leading to the observed phenotype.

Preliminary *in vitro* experiments suggest that the presence of TNF increases the suppressive function of regulatory T cells leading to reduced proliferation of MOG-specific 2D2 T cells. These findings are in line with recent studies that have proposed that TNF may play a role in

regulatory T cell (Treg) proliferation, survival and function. The mechanism underlying this modulation and the role of TNF on the dynamics of Treg mediated autoimmune suppression *in vivo* has not been addressed properly. We are therefore planning *in vivo* studies that will help to better understand the specific effects of TNF on Tregs during EAE.

Characterization of B lymphocyte subpopulations in Natalizumab treated MS patients. From phenotype to function

Claudia Sievers, Maria Meira, Francine Hoffmann, Hedwig Wariwoda, Heidi Bodmer, Tobias Derfuss, Ludwig Kappos, Raija LP Lindberg

Clinical Neuroimmunology, Departments of Biomedicine and Neurology, University Hospital Basel, Switzerland

Background: Peripheral T and B cells play an important role in immune pathogenesis of multiple sclerosis (MS). Natalizumab (NTZ), one of the current treatments, inhibits efficiently the migration of these cells over the blood-brain-barrier (BBB) by blocking alpha 4 Integrin (CD49d). However, not only binding but also expression of CD49d is altered by NTZ in various lymphocyte subpopulations. Furthermore, we have shown that NTZ alters microRNA (miRNA) expression in B cells [1] and B cell subsets. Several members of the miR-17-92 and miR-106b-25 clusters are deregulated in naïve and memory B cells. These miRNA clusters regulate functionally interrelated pathways involved in apoptosis and cell survival.

Objectives: 1) Analysis of potential target mRNA and protein expression of deregulated miRNAs in total, naive and memory B cells. 2) Characterization of transmigration properties of B cells and various B cell subsets.

Methods: Total, naïve and memory B-cells were separated from peripheral blood of untreated (UT) and NTZ treated RRMS patients and healthy volunteers (HVs). B cell purity was assessed with flow cytometry. Total RNA was isolated with miRNeasy Mini Kit. mRNA expression was analyzed using RT-PCR based assays. Protein expression was detected by intracellular FACS- staining and by Western Blot analysis. The hCMEC/D3 cell line was used as a BBB model. CXCL13 was applied as B cell specific chemokine.

Results: We found decreased expression of miR181a, miR15 and miR16 in total, naive and memory B cells in NTZ treated patients compared to UT patients. Consequently, mRNA and protein expression of the target, Bim, was increased. However, the expression of BCL2, regulated by the same miRNAs, was already increased in UT patients compared with HV, while NTZ treatment had no further influence on BCL2 expression. Furthermore, although the expression of miR93, miR17 and miR181a was decreased in total and naive B cells, the expression of PTEN, a target of these miRNAs, was not altered. Our preliminary transmigration assays show the expected decrease in B cell migration in NTZ treated patients in response to CXCL13. Furthermore, the initial results suggest differences in migration capacity of naive and memory B cells in MS patients compared with HVs. In addition, NTZ seems to have a specific effect on various subsets of B cells. However, further validation is needed.

Discussion and conclusion: We hypothesize that increased numbers of B-cells, especially memory-B cells in NTZ treated patients compared to UT patients and HVs, might be due to changes in apoptosis and/or survival of those cells. However, our present data of deregulated miRNAs and their potential target mRNA expression doesn't support this assumption. Namely, decreased expression of miR-15, -16 and -181a in total, naive and memory B cells of NTZ treated patients correlates with an increased expression of Bim, proapoptotic protein of the intrinsic pathway, and therefore would rather suggest increased apoptosis. Interestingly, the expression of BCL2, an antiapoptotic molecule, regulated by the same miRNAs, is increased in untreated patients compared with HVs, but is not influenced by NTZ treatment. In addition, the expression of PTEN, which is an inhibitor of PI3K pathway, which promotes cell cycle progression and inhibits apoptosis, is not altered in B cells of NTZ patients. Therefore, increased numbers of B cells in NTZ treated patients, is not due to increased survival of the cells but indeed likely due to decreased migration over BBB. Interestingly, our preliminary results suggest cell subset specific properties of transmigration, which is also treatment duration dependent.

1. Sievers, C., Meira, M., Hoffmann, F., Fontoura, P., Kappos, L. and Lindberg, R. L. P., Altered microRNA expression in B lymphocytes in multiple sclerosis: Towards a better understanding of treatment effects. *Clinical Immunology* 2012. 144: 70-79.

The role of brain barrier tight junctions in the pathogenesis of experimental autoimmune encephalomyelitis

Silvia Tietz[1], Pascale Baden[1], Julia Michel[1], Elisabetta Dejana[2], Mikio Furuse[3], Beat Imhof[4], Urban Deutsch[1], Britta Engelhardt[1]

[1] *Theodor Kocher Institute, University of Bern*

[2] *IFOM, Milan, Italy*

[3] *Division of Cell Biology, Kobe University Graduate School of Medicine, Kobe, Japan*

[4] *Centre Médicale Universitaire, Geneva*

During neurological disorders such as multiple sclerosis (MS) or its animal model experimental autoimmune encephalomyelitis (EAE), focal loss of blood-brain barrier (BBB) integrity is observed and associated with the formation of inflammatory lesions inside the central nervous system (CNS). Besides occludin, other transmembrane proteins are localized in BBB tight junctions including members of the claudin family (claudin-3, claudin-5 and claudin-12) and the junctional adhesion molecule (JAM) family (JAM-A, JAM-B, JAM-C). A specific contribution of BBB tight junctions in EAE pathogenesis has been proven by our previous observations that loss of the BBB tight junction protein claudin-3 correlates with immune cell infiltration into the CNS and BBB leakiness. JAM-A and JAM-B have been implicated in leukocyte adhesion and diapedesis across endothelial barriers of peripheral vascular beds. JAM-A is expressed by different leukocyte subsets while JAM-B is exclusively expressed by endothelial cells. We detected an up-regulation of JAM-A during inflammatory conditions of $MOG_{aa35-55}$-induced EAE in the BBB vasculature and at sites of leukocyte infiltration JAM-A was exposed on the luminal side of BBB endothelial cells, indicating an active involvement in leukocyte extravasation. In JAM-$A^{-/-}$ mice $MOG_{aa35-55}$-induced EAE was milder and delayed accompalished by a reduction of infiltrating leukocytes into the spinal cord compared to wild-type littermates. Recently, JAM-B has been described as an alternative α□-integrin ligand. Considering the dominant role for α□ /$-integrin in T cell trafficking to the CNS during EAE we hypothesize that JAM-B might provide an additional vascular ligand for α□ /$-integrin and and may thus contribute to immune cell trafficking across the BBB and thus to EAE pathogenesis. EAE studies using JAM-$B^{-/-}$ suggest development of ameliorated EAE in JAM-$B^{-/-}$ C57BL/6 mice when compared to wild-type littermate controls. So far our data suggest that a lack of claudin-3 results in an aggravation of EAE. Moreover, our studies demonstrate a role for JAM-A in EAE pathogenesis by specifi-

cally regulating monocyte but not T cell recruitment across the inflamed BBB and JAM-B-deficiency appears to be beneficial in the pathogenesis of EAE.

Detailed effects of prolonged-release fampridine on ambulatory function in patients with multiple sclerosis (FAMPKIN-Study)

Björn Zörner, Katja Reuter, Linard Filli, Adam Czaplinski, Lilla Lörincz, David Weller, Tabea Sutter, Melinda Farkas, Sandra Kapitza, Michael Linnebank

Department of Neurology, University Hospital Zurich

Previous studies reported positive effects of fampridine (4-aminopyridine) on gait velocity in a subset of MS patients. Fampridine is a blocker of voltage-gated potassium channels and thus can induce improved signal conduction in demyelinated nerve fibers.

The objective of the FAMPKIN study (http://clinicaltrials.gov) was to characterize the detailed effects of prolonged-release (PR) fampridine on different modalities of walking function including muscle strength, stability and coordination, as well as on physical activity during everyday life. The study was designed as phase II, randomized, double-blind, and placebo-controlled cross-over study assessing gait function during two double-blind fampridine or placebo treatment phases (each 6 weeks). Walking and physical activity was investigated using different clinical tests, accelerometer devices, questionnaires and detailed kinematic gait analysis. 55 patients with relapsing-remitting, primary- and secondary-progressive MS were analyzed (34 women, 21 men; age 48.4 +/- 9.7 years; median EDSS = 4.5). Patients were categorized into responder and non-responder groups according to the criteria used previously (Goodman *et al.*, 2009). 31% (n=17) of all participants were identified as fampridine-responders, 5% (n=3) were placebo-responders, and 9% (n=5) met the responder criteria in both phases.

Gait velocity (timed-25-foot walk) was significantly increased during the fampridine treatment compared to the placebo treatment (+5.8% in the total population (n=55), +12.3% in pure fampridine-responders (n=17), +3.3% in non-responders (n=29)). Walking endurance (6-minute walk test) was substantially ameliorated by fampridine by 8.5% in the total population, 13.5% in pure fampridine-responders, and 6.1% in the non-responder group. The

Timed Up and Go Test (TUG), the Berg Balance Scale (BBS) and the Dynamic Gait Index (DGI), did not reveal significant improvements induced by fampridine. Measures of physical activity during everyday life showed a significant, fampridine-induced increase of activity in the subgroup of pure fampridine-responders, whereas physical activity was unchanged in the total population and the subgroup of non-responders.

Gait profiles consisting of multiple kinematic and kinetic parameters demonstrated heterogeneous, fampridine-induced gait modifications. The differential changes in the gait pattern among patients most likely reflect individual improvements of the gait pattern depending on the specific deficits of each patient.

The present study demonstrates beneficial effects of fampridine on different aspects of locomotion and indicates the relevance of this symptomatic treatment for a subset of patients with MS.