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ABSTRACTS

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ORAL PRESENTATIONS

Characterization of encephalitogenic CD8+ T cells in an animal model of multiple sclerosis

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Multiple sclerosis (MS) is the most common inflammatory demyelinating disorder of the central nervous system (CNS) causing disability in young adults. Histopathological studies suggested that CD8+ cytotoxic T lymphocytes (CTLs) may contribute to CNS tissue damage in MS. However, most MS animal models are mostly mediated 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). We took advantage of this new animal model, to monitor the microbial capacity of two recombinant pathogens expressing OVA (Lm-OVA and LCMV-OVA) to elicit an encephalitogenic CTLs response. Adoptively transferred OVA-specific CTLs (OT-1) expanded to a similar extent showing clonal differentiation into effector CTLs in ODC-OVA mice upon peripheral infection with either Lm-OVA or LCMV-OVA. However, EAE disease was only observed upon LCMV-OVA infection.

To identify a gene expression signature that may characterise encephalitogenic brain invading CTLs, we performed a transcriptome analysis on FACS-sorted brain infiltrating OT-1 cells after LCMV-OVA or Lm-OVA challenge. We found that the core transcriptional signature was similar in the two experimental groups, but some genes were differentially regulated in CTLs in diseased versus non-diseased animals. The implication of these differentially expressed genes was further investigated in functional readouts and correlated to the encephalitogenic property of CTLs. Thus, our study may provide new insights into the underlying mechanisms that govern functional and transcriptional regulation of an encephalitogenic CTLs.

Oxysterols and human memory T lymphocytes: an attractive story

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Aims. Oxysterols, hydroxylated cholesterol metabolites, modulate the immune response and promote inflammation (1). We are interested in studying the role of the oxysterol 7α25-hydroxycholesterol (7α25-OHC), the strongest ligand of the Epstein-Barr virus-induced G-protein coupled receptor 2 (EBI2). Using the experimental autoimmune encephalomyelitis, an animal model for multiple sclerosis (MS), we previously showed that memory CD4+ T lymphocytes migrate specifically in response to 7α25-OHC via EBI2. Furthermore EBI2-deficient lymphocytes depict delayed migration to the central nervous system compared to their wild type counterparts (2). However, the expression and the role of EBI2 in human lymphocytes during Multiple Sclerosis (MS) have not been studied. We now propose to study EBI2 expression and function in human lymphocytes in healthy donors and MS patients.

Methods. EBI2 expression on human peripheral blood mononuclear cells was measured by flow cytometry using a specific anti-human EBI2 antibody. The function of EBI2 in cell migration was assessed using a transwell assay.

Results. We observed maximal EBI2 expression on memory CD4+ T cells; memory subsets of B and CD8+ T cells also depicted a modestly increased EBI2 expression compared to naive populations. Transwell migration assay experiments showed maximal migration of memory CD4+ T cells in response to 7α25-OHC. Even if globally less responsive to 7α25-OHC than the latter, memory subsets of B and CD8+ T cells were also found to migrate more strenuously than their naive counterparts. This chemotaxis was specific to EBI2 as selective EBI2 inhibition unequivocally abrogated migration. Finally, EBI2 expression and migration pattern are modified during MS compared to healthy donors.

Conclusion. These data suggest an important role for EBI2 in human T cell migration. Selective targeting of immune cell trafficking has become an important tool in the clinical setting to dampen autoimmunity, in particular during MS. Uncovering the role of EBI2 in T cell trafficking, in particular its ability to direct human CD4+ T cell chemotaxis, may open new avenues for understanding the pathogenesis of autoimmunity and may promote novel therapeutic approaches.

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Endothelial ALCAM (CD166) is not required for encephalitogenic T cell migration across the blood-brain barrier

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Aim. Extravasation of circulating CD4+ effector/memory T cells (TEM cells) across the blood-brain barrier (BBB) is a tightly regulated multi-step process. Each step of the extravasation cascade is mediated by different adhesion and signalling molecules expressed on the TEM cell and on the brain endothelial cell. Our previous work has shown essential but differential roles of vascular cell adhesion molecule (VCAM)-1, endothelial intercellular adhesion molecule (ICAM)-1 and ICAM-2 for the shear resistant arrest, crawling and diapedesis of TEM cells. Activated leukocyte cell adhesion molecule (ALCAM) is another endothelial cell adhesion molecule expressed on the human BBB. The observation that an anti-ALCAM antibody ameliorated experimental autoimmune encephalomyelitis (EAE) disease course in the mouse, supported a role of ALCAM in TEM cell trafficking to the CNS. This prompted us to investigate the role of endothelial ALCAM for the extravasation of CD4+ TEM cells across the mouse BBB.

Methods. Primary mouse brain microvascular endothelial cells (pMBMECs) from ALCAM-knockout (ko) or wild type (wt) C57BL/6J mice as in vitro BBB model. T cell diapedesis under static conditions. In vitro live cell imaging under physiological flow. Quantitative polymerase chain reaction and Western Blotting to assess ALCAM expression. Immunofluorescences of histological sections of the mouse or human brain or spinal cord. Experimental autoimmune encephalomyelitis (EAE).

Results. Diapedesis of TEM cells across unstimulated ALCAM-ko pMBMECs was reduced compared to wt pMBMECs. In contrast, under physiological flow conditions the dynamic interaction of CD4+ TEM cells with ALCAM-ko or wild type pMBMECs remained comparable. Detectable ALCAM mRNA levels in wild type pMBMECs did not translate into detectable ALCAM protein levels under unstimulated or cytokine stimulated conditions of the pMBMECs. However, ALCAM protein was readily detected in mouse brain and spinal cord lysates confirming presence of ALCAM in the CNS. Immunofluorescence staining of brain or spinal cord sections from wild type mice proved ALCAM protein below detection limit on parenchymal CNS vessels in the mouse. Finally, EAE in ALCAM-ko mice was rather aggravated when compared to wild-type littermates.

Conclusion. Our data point to a role of ALCAM in autoimmune CNS inflammation of the mouse that is different from mediating the migration of encephalitogenic CD4+ TEM cells across the BBB.

Spinal cord gray matter atrophy – a biomarker for MS progression

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Aims. In Multiple Sclerosis (MS) cerebral gray matter (GM) atrophy correlates more strongly with disability than does white matter (WM) atrophy. Advances in phase sensitive inversion recovery (PSIR) imaging now enable specific assessments of the spinal cord (SC) GM and WM. The goal of this study was to assess GM and WM areas in the cervical and thoracic SC and their relationship with disability and disease type in MS.

Methods. 142 MS patients (25--75 years, 86 women) and 20 controls were scanned at 3T. Axial 2D- PSIR images were acquired at the disc levels C2/3, C3/4, T8/9 and T9/10. Total cord areas (TCA) were segmented semi-automatically. SC GM areas were segmented manually. Differences in areas between groups were assessed with age and sex as covariates. The relative contribution of demographics, clinical characteristics, and PSIR-derived measures to Expanded Disability Status Scale (EDSS) variability were investigated using analyses of relative importance of regressors in a linear model. Receiver operating characteristic (ROC) curves were compiled to assess sensitivity and specificity for predicting a progressive disease course based on the variables with the highest contribution to EDSS.

Results. In the cervical and thoracic SC relapsing MS patients had significantly smaller GM areas than controls ($p < 0.001$ at C2/3; $p=0.003$ at T8/9; $p=0.011$ at T9/10), but had no significant difference in either the SC WM area or TCA. Progressive MS patients showed smaller GM areas ($p < 0.001$ at all levels) and TCAs ($p < 0.001$ at C2/3, C3/4, T8/9; $p=0.004$ at T9/10) compared to relapsing MS patients. In multivariable models (including SC WM areas and T2-lesion number, brain WM volumes, T1- and FLAIR-lesion loads, age, sex, disease duration) cervical SC GM area had the strongest correlation with EDSS followed next by thoracic SC GM area and brain GM volume. The areas under the ROC curve were 0.68, 0.84 and 0.87 for the prediction of a progressive course based on logistic models with 1) brain GM volume, 2) cervical GM area and 3) cervical, thoracic GM areas and brain GM volume as predictors, respectively.

Conclusions. This study provides evidence for the clinical impact of cord GM atrophy in MS, as measured in vivo by PSIR imaging. GM atrophy is detectable at multiple cord levels in the absence of WM atrophy in relapsing MS. It is more pronounced in progressive MS than relapsing MS and contributes more to patient disability than SC WM or brain GM atrophy.

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Nogo-A-antibodies as a potential novel therapy for Multiple Sclerosis

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Aims. The two hallmarks of chronic Multiple Sclerosis (MS) lesions are (1) absence of significant spontaneous remyelination and (2) primary as well as secondary neurodegeneration due to chronic axonal demyelination and inflammation. Both of these pathogenic characteristics may be influenced by the presence of inhibitory factors preventing myelin and neuronal repair. A factor potentially involved is the myelin-associated protein Nogo-A which is known as an inhibitor of neurite outgrowth. Several lines of evidence support the involvement of Nogo-A in the pathogenesis of MS: First, Nogo-A-antibody treated mice with Experimental Autoimmune Encephalomyelitis (EAE) show improved functional outcome and neuronal survival (Karnezis et al., Nat. Neurosci., 2004). Second, Nogo-A deficient mice show enhanced myelinogenic potential and remyelination after lysolecithin-induced demyelination (Chong et al., PNAS, 2012). However, several questions remain to be answered: First, how do Nogo-A-antibodies mediate enhanced functional recovery and second, does pharmacological blockade of Nogo-A by function blocking antibodies against Nogo-A improve remyelination? Therefore, we aim at investigating in this study the potential of anti-Nogo-A immunotherapy to enhance neuronal regeneration and remyelination in two animal models for MS.

Methods and results. After induction of a focal EAE lesion in the dorsal funiculus of the cervical spinal cord of rats, improved recovery of forelimb function was observed in the anti-Nogo-A treated group. By anterograde BDA tracing of the corticospinal tract in pilot experiments, the anti-Nogo-A group showed increased neuronal sprouting of the injured tract. Moreover, rats treated with anti-Nogo-A antibodies showed enhanced remyelination after lysolecithin-induced demyelination of the cervical spinal cord as demonstrated by an increased count of remyelinated axon within the lesion.

Conclusions. These preliminary findings hint towards Nogo-A-antibodies as a possible new treatment approach for MS. This may be particularly interesting for treating the chronic progressive phase of MS where the neurodegeneration and remyelination failure are hallmarks.

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IDO competent plasmacytoid dendritic cells regulate CNS autoimmunity

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Dendritic cells represent a heterogeneous pool of professional Antigen Presenting cells (APCs) playing a pivotal role in Immunity. Conventional dendritic cells (cDC) function as key APC during both priming and effector phases of EAE. To define the contribution of MHCII-mediated Ag presentation by plasmacytoid DC (pDC), we have studied the development of EAE in mice exhibiting a selective loss of MHCII expression by pDCs. We have previously shown that MHCII expression by pDCs results in encephalitogenic T cell priming inhibition and promotes the development of regulatory T cells (Treg) in secondary lymphoid tissues.

The present work investigates the mechanisms underlying pDC-mediated Treg expansion and EAE protection. Using mice in which Treg can be selectively depleted, we show that EAE development is severely exacerbated in absence of Treg during the priming phase of the disease, to similar extent compared to mice lacking MHCII expression by pDCs. Aggravated EAE symptoms correlated with increased encephalitogenic T cell priming in draining lymph nodes (LN). We then addressed the possible mechanisms accounting for pDC-mediated Treg development. We observed that the expression of Indoleamine-2,3-dioxygenase (IDO), which has been shown to play a role in Treg generation and maintenance and to be link to pDC tolerogenesis, is preferentially expressed in pDCs compared to other cell types in LN. Importantly, we demonstrate that IDO expression by pDCs is mandatory to confer suppressive functions to Treg and, consequently, to dampen EAE severity.

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

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Aims. The aim of our project is to elucidate the role of Btn2a2, a distant relative of the B7 family of costimulatory molecules, in the modulation of T cell responses. This could potentially lead to new therapeutic applications for autoimmune conditions and inflammatory disorders such as multiple sclerosis (MS).

Methods. Approaches included gene-expression studies, generation Btn2a2-/- mice, analysis of in vivo CD4+ T cell responses in Btn2a2-/- mice, analysis of the susceptibility of Btn2a2-/- mice to MOG-induced experimental autoimmune encephalomyelitis (EAE), an animal model for MS, and in vitro T cell activation studies performed in the presence of a Btn2a2-Ig fusion protein.

Results. Human BTN2A2 and mouse Btn2a2 genes were found to be co-regulated with MHC class II (MHCII) genes in antigen presenting cells (APCs) and IFN-γ induced cells, suggesting a role in MHCII-mediated antigen presentation to CD4+ T cells. Generation and analysis of Btn2a2-/- mice confirmed this. Immunization experiments demonstrated that CD4+ T helper (Th) cell responses were markedly enhanced in Btn2a2-/- mice. Accordingly, EAE, a CD4+ Th-cell mediated autoimmune disease of the central nervous system, was strongly exacerbated in Btn2a2-/- mice. Btn2a2-/- mice exhibited accelerated disease onset, higher cumulative and maximum disease scores, and greater disease incidence. Exacerbated disease was associated with increased infiltration of the spinal cord by pathogenic IFN-γ and IL-17 producing Th cells. Conversely, frequencies of infiltrating CD4+Foxp3+ regulatory T cells (Treg) were reduced. EAE experiments performed with reciprocal bone marrow chimeras (Btn2a2-/- into WT and WT into Btn2a2-/-) demonstrated that disease exacerbation was due to loss of Btn2a2 expression by cells of hematopoietic origin. TCR-transgenic T cell transfer experiments indicated that Btn2a2 expression by APCs modulates CD4+ T cell responses in vivo, leading to dampened Th responses in favor of increased Treg expansion. Finally, in vitro T cell activation assays performed in the presence of a Btn2a2-Ig fusion protein confirmed that Btn2a2 inhibits CD4+ T cell activation and proliferation, impairs Th cell differentiation and enhances Treg development.

Conclusions. Our results demonstrate that Btn2a2 is a novel negative regulator of T cell responses, and that it protects against the development of T cell mediated neuroinflammation.

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The Neurotrophic Hepatocyte Growth Factor Negatively Regulates The Cytotoxic T-Lymphocyte Activity of Murine CD8+ T cells

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Aim(s). Controlling the mechanisms that govern the functions of encephalitogenic T cells is critical in the context of Multiple Sclerosis (MS) intervention. We recently showed that hepatocyte growth factor (HGF), a potent neuroprotective factor, restrains CD4+ T cell-mediated autoimmune neuroinflammation at least in part through the generation of tolerogenic dendritic cells (DCs) (1, 2). Due to the increasing appreciation in MS for the role of CD8+ T cells, also known as cytotoxic T lymphocytes (CTLs), we chose to further investigate whether cytolytic CTL responses can be modulated by HGF.

Methods. Effector CD8+ T cells from gp100-specific T cell receptor transgenic (Pmel-1) mice were generated in vitro. The phenotypic characteristics of CTLs were analyzed by flow cytometry. The cytolytic function of effector CD8+ T cells was examined using established models of CTL-mediated killing.

Results. We observed that HGF restrained the generation of effector cytotoxic CD8+ T cells from naïve splenocytes. Importantly, CTLs generated in the presence of HGF showed a lower level of cytolytic activity, as measured by specific in vitro and in vivo killing of antigen-pulsed target cells, including primary cortical cells. Mechanistically, HGF reduced the production of inflammatory cytokines and cytolytic enzymes by CTLs, including interferon-γ, tumor necrosis factor, perforin, and granzyme B. While HGF further lessened the expression of membrane-bound death receptor Fas ligand, a non-redundant lytic mechanism with cytolytic granule release in CTL-mediated killing, treatment of CD8+ T cells with concanamycin A, an inhibitor of the perforin-mediated cytotoxic pathway, abrogated CTL cytotoxicity indicating that blockade of the perforin-dependent killing is a major mechanism by which HGF diminished cytolysis of target cells. Of specific importance, similar results were obtained when HGF-treated DCs were cultured with naïve purified CD8+ T cells.

Conclusions. These results indicate that HGF limits the effector function of CTLs via DCs. Complementary to its impact on CD4+ T-cell CNS autoimmunity and myelin repair, our findings further suggest that HGF treatment could be exploited to control CD8+ T-cell-mediated, MHC I-restricted autoimmune dysfunctions such as MS.

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Extending the spectrum of anti-MOG antibody positive inflammatory CNS disease: Results from the Swiss Lupus Cohort Study

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Background. Myelin oligodendrocyte glycoprotein (MOG) has long been controversially discussed as potential autoantigen in multiple sclerosis (MS). Only recently, with the use of cell-based assays, we and others have shown that anti-MOG antibodies are indeed present in about 25% of children with MS and acute demyelinating encephalomyelitis (ADEM) and that antibody levels correlate with the disease course. More recently, we among others identified anti-MOG IgG (immunoglobulin G) in a subgroup of aquaporin-4 (AQP4)-seronegative patients with neuromyelitis optica spectrum disease (NMOSD) presenting with a distinct, more benign clinical phenotype as compared to AQP4-seropositive patients. The frequently observed coexistence of disease-specific anti-AQP4 IgG with other autoantibodies, including antinuclear antibody and antibodies to extractable nuclear antigens, in NMOSD patients raises the question of how the clinical syndrome of NMO correlates with a systemic rheumatologic disease in these patients. Previous studies investigating the relationship between NMOSD and systemic lupus erythematosus (SLE) have pointed towards an overlap syndrome between SLE and NMO in a subgroup of patients which is most likely caused by autoantibody-mediated demyelinating lesions rather than cerebral vasculitis. However, the target antigen(s) up to now remains elusive.

Methods. We analyzed the presence of anti-MOG IgG in a large, blinded, unbiased cohort of SLE patients from the Swiss Lupus Cohort (n=173) at baseline and follow-up. In addition, antibodies against AQP4, neurofascin and a variety of neuropil antibodies will be tested in all baseline samples.

Results. Of the 173 SLE patients included in the study, 15 patients (8.7%) were tested positive for anti-MOG IgG during the disease course with a female preponderance (12/15, 80%). Follow-up samples were available in 10 patients, of which 6 showed fluctuating antibody titers. 3 of the 15 patients developed antibodies during the disease course (after 3 and 5 years) and one patient lost the IgG at follow-up. The clinical and MRI data as well as the correlation to other autoantibodies tested will be presented at the meeting.

Conclusion. Anti-MOG IgG can be detected in a subgroup of SLE patients with and without apparent neurological deficits. Thus, anti-MOG IgG could serve as a potential biomarker to identify SLE patients with an overlapping demyelinating syndrome and possibly lead to a more targeted therapy (i.e. B cell depletion).

POSTERS

P02

Development of a CNS in vitro model based on induced pluripotent stem cells derived from blood of multiple sclerosis patients

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Aim. In multiple sclerosis (MS), the mechanisms involving the interactions between the immune system and the central nervous system (CNS) are still poorly understood. This situation is partly due to the limited access to MS patients CNS samples. To overcome this issue, we propose to build an in vitro model based on induced pluripotent stem cells (iPSC) derived from blood cells of MS patients. These iPSC will be differentiated into neurons, astrocytes and oligodendrocytes to study the effects of the immune system of MS patients on autologous CNS cells.

Methods. Peripheral blood mononuclear cells (PBMC) are nucleofected with episomes coding for transcription factors OCT3/4, SOX2, KLF4, c-Myc and LIN28 (Yamanaka cocktail) and cultured in conditions adapted to iPSC cell culture. Stable and characterized iPSC cultures that can be differentiated into neural stem cells (NSC) are obtained after 5-6 months. NSC are differentiated by culturing iPSC colonies in neural induction conditions and expanded by addition of basic fibroblast growth factor and epithelial growth factor. To generate neurons, NSC are cultured with brain-derived neutrophic factor and terminally differentiated by removal of all growth factors. For differentiation in astrocytes, NSC are cultured with ciliary neutrotrophic factor or bone morphogenic protein 4.

Results. Up to now, we have generated at least 5 iPSC clones from PBMC of each of the 6 subjects enrolled (5 MS patients and 1 healthy control). These iPSC formed typical homogenous round-shaped colonies composed of small round cells, expressed pluripotency markers and had the capacity to differentiate into the 3 embryonic germ layers. Neurons as identified by a typical neuronal morphology, an dendrite network, and a MAP2 staining were 90% pure. Astrocytes are recognized by their star-like morphology and positive GFAP staining. Up to now, the maximal degree of purity was 46%.

Conclusion. These preliminary data show that it is possible to derive CNS cells from the PBMC. We are currently working on improving the degree of purity of astrocytes cultures to reach >90% GFAP+ cells, as well as developing the technique to obtain oligodendrocytes. Once fully developed, this “brain in a dish” system will be of a high value to study the interactions between autoreactive immune T cells and autologous CNS cells. This model should also serve as a pre-clinical screening assay to evaluate the neuroregenerative potential of candidate compounds in humans.

P03

Mechanisms distinct from those used by CD4+ TEM cells regulate CD8+ TEM cell migration across the blood-brain barrier under flow conditions in vitro

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T cell trafficking into the central nervous system (CNS) is a crucial step in the pathogenesis of multiple sclerosis (MS) and is controlled by the highly specialized endothelial cells forming the blood-brain barrier (BBB). The molecular mechanisms mediating the multi-step extravasation of CD4+ effector T cells across the BBB are well described. Although there is accumulating evidence for an involvement of CD8+ T cells in MS pathogenesis it remains to be shown if they use similar or different cues from CD4+ T cells for crossing the BBB. To do so, we used primary mouse brain microvascular endothelial cells (pMBMECs) as an in vitro model of the BBB. A homemade flow chamber combined with a high magnification live cell imaging allowed us to visualize the interaction of CD8+ TEM with the BBB. By side by side comparing the interaction of CD8+ TEM cells versus CD4+ TEM cells with the BBB under physiological flow in vitro, we could observe that CD8+ TEM cells arrested almost 3-fold better than CD4+ TEM cells on pMBMECs under non-inflammatory and inflammatory conditions.

Assessment of the dynamic behavior of these arrested T cells revealed that while for CD4+ TEM cells diapedesis required prior crawling, the vast majority of CD8+ TEM cells remained stationary before crossing. Diapedesis of CD8+ TEM cells was 2-fold higher than that of CD4+ TEM cells. Interestingly, while CD8+ TEM cells preferentially crossed the endothelium via a transcellular pathway, CD4+ TEM cell diapedesis was mostly observed via the the paracellular pathway. On pMBMECs lacking ICAM-1 and ICAM-2 arrest of CD8+ TEM cells was almost abolished while the arrest of CD4+ TEM was not significantly affected. Lack of ICAM-1 and ICAM-2 also led to a defect in crawling of both cells. Nevertheless, CD8+ TEM cells were still able to cross the BBB better than CD4+ TEM cells. In contrast to CD4+ T cell migration, Ag cross-presentation by brain endothelium may influence the migration of CD8+ T cells. We then have started to study the role of Ag presentation by the endothelium in mediating CD8+ TEM cell diapedesis focuing on defining which step of the multi-step process might be affected.

Our study highlights that cellular and molecular mechanisms mediating CD8+ TEM cell extravasation across the BBB are distinct from those regulating CD4+ TEM cells in vitro. This opens new possibilities for subset-specific therapeutic targeting of T cell migration across the BBB during neuroinflammation like MS.

P04

Characterisation of cells present in cerebrospinal fluid of MS patients and controls using Time-of-Flight mass cytometry

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Background. A central feature of multiple sclerosis is the presence of immune cells in the brain, which are likely to be involved in the disease's debilitating pathology. A better understanding of these cells therefore promises to be valuable in understanding the disease. Since cells in the brain parenchyma are generally inaccessible to examination, the most closely related population, i.e., those found in the cerebrospinal fluid (CSF), are important targets for investigation. This requires techniques to maximise the amount of information obtained from small numbers of cells, and one attractive candidate is the recently developed cytometry by time-of-flight (CyTOF), which simultaneously can measure more than thirty phenotypic properties on each cell.

Aim. The aim of the project was to develop a CyTOF protocol to measure the frequencies and phenotypes of T cells in CSF and in peripheral blood. Methods Peripheral blood and CSF were collected during routine diagnostic procedures from informed, consenting individuals. Mononuclear cells (PBMCs) were separated from blood by density gradient centrifugation, and CSF cells by centrifugation. PBMCs and CSF cells were separately prelabelled to enable separation of the two populations, then mixed, fixed, permeabilized and labeled with lanthanide isotope-conjugated antibodies against a panel of markers (including HLA-DR, CD3, CD4, CD8, CD11b, CD11c, CD14, CD16, CD20, CD27, CD45RO, CD56, CD138, IgM, BAFFR, MCAM, CCR5 , FoxP3, Tbet, CD14, CD11b, CD16, BAFFR, CD11c, CD45RO, CD27, HELIOS, GM-CSF,, IgM, IL-4, IL-17A, IFNg, MCAM , TNFa, GM-CSF, CCR5 , CD138 , IL-17A, CD45). To examine cytokine production, cells were stimulated with phorbol ester and ionomycin for 3 h in the presence of protein transport inhibitors, then fixed and labeled. Lanthanide-labels were detected by CyTOF and data analysed using Cytobank software.

Results. 3,000 to 20,000 CSF cells were obtained from each patient, and more than twenty constitutively expressed antigens could be reliably labelled and measured simultaneously on these cells. Using the SPADE algorithm from Cytobank, all relevant immune cell populations could be identified. Establishment of a protocol for cell activation and intracellular cytokine labeling of CSF cells is ongoing.

Conclusion. CyTOF offers a promising approach to detailed phenotyping of the small numbers of cells found in CSF; further work is needed to enable the characterisation of their cytokine profiles.

P05

Increased ex-vivo antigen presentation profile of B cells in multiple sclerosis

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Aims. Multiple sclerosis (MS) is thought to be triggered by environmental agents such as Epstein-Barr virus (EBV) in genetically-susceptible persons, a combination which will lead to autoimmunity but the precise mechanism remains enigmatic. Few studies have examined whether the innate immune response is dysregulated in MS patients. Here, we proposed to examine what stands upstream from T cell activation studying in detail the phenotype and activation level of different antigen-presenting cells (B cells and monocytes).

Methods. We enrolled 98 study subjects including patients suffering from relapsing-remitting (RR), secondary-progressive (SP), primary-progressive (PP) MS, other inflammatory neurological diseases (OIND) and healthy controls (HC). On the day of blood draw, monocytes and B cells were isolated and their ex vivo profile of activation, as reflected by surface expression of CD40, CD80, CD83, CD86, and HLA-DR was assessed by flow cytometry. Cells were then stimulated overnight using either specific ligands of toll-like receptors (TLR) 1 to 9 or EBV particles. Cytokine and chemokine secretion profiles (GM-CSF, IFN-a, IL-1a, IL-1b, IL-6, IL-10, IL-23p19, TNF-a) were finally assessed by Luminex assay in recovered supernatants. Following log- or rank-transformation of the measured values, differences among groups and stimulations were assessed in a linear model framework, adjusting for age and gender.

Results. We demonstrate that MS patients exhibit a significant increased expression of HLA-DR and CD40 at the surface of monocytes, and mostly of B cells, especially during relapses. No such increase of HLA-DR or CD40 was seen in OIND patients. Interestingly, this phenotype is associated with a decreased basal secretion of IL-6 and TNF-a by B cells of RR-MS, and of IL-1b by monocytes of SP and PP-MS patients. Upon stimulation, there is a rescue of the level of these cytokines, which reach similar levels in all conditions tested.

Conclusions. These data clearly suggest that the antigen presentation function of B cells and to a lesser extent of monocytes as well as their cytokine content is dysregulated in MS, but with different profiles depending on the stage of the disease.

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P06

Longitudinal analysis of topographic VEP in relapsing-remitting patients with multiple sclerosis

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Background. Visual evoked potentials (VEP) are used to evaluate therapies aiming at remyelinization in multiple sclerosis (MS) (Cadavid et al. AAN 2015). Topographic VEP analysis is more sensitive than conventional VEP when the configuration is pathologically altered since the P100 component is determined automatically (Hardmeier et al. 2014). Objective: To evaluate whether topographic VEP measures can detect subclinical change in MS eyes with and without previous optic neuritis (ON).

Methods. 57 relapsing-remitting MS patients (median age: 39 years, median EDSS: 2.0, interquartile range: 1.5-3.0) and 32 healthy controls (HC) had VEP at years 0, 1 and 2 using a 204 electrode array during full-field checkerboard stimulation. The topography of the field distribution was used to determine the P100 component in relation to a reference topography yielding a latency (Lat), amplitude (Amp) and correlation (Fit) measure for left (L) and right (R) eyes.

Results. Cross-sectional comparisons between HC and MS eyes with previous ON (MS-ON; n=16 L, n=17 R) and without (MS-nON) showed similar results at all time points: Lat discriminated best between groups (median value over years 0-2 and both eyes, HC: 104ms, MS-nON: 112ms, MS-ON: 134 ms; $p<0.0001$ for all comparisons), Fit (HC: 0.96, MS-nON: 0.94, MS-ON: 0.87; p-value range: not significant to $p<0.0001$) and Amp (HC: 1.43, MS-nON: 1.17, MS-ON: 0.80; $p<0.05$ to $p<0.01$) to a lesser extent. Test-retest-reliability in HC was highest for Lat (intraclass-correlation coefficient [ICC]: 0.91 R, 0.95 L) and Amp (ICC : 0.80 R, 0.83 L) but poor for Fit (ICC : 0.56 R, 0.51 L). Repeated measures ANOVA showed change over time in MS-ON (Fit R, $p<0.01$); however, visual acuity, EDSS as well as VEP Lat and Amp remained stable.

Conclusion. Topographically determined P100 latency is reliable and valid but reveals no change over two years in this clinically stable cohort of MS patients. The change in the topographic correlation measure (Fit) in eyes with previous ON over time may relate to subclincial changes; however, this has to be confirmed by longer follow-up. The main advantage of topographic analysis lies in an automated determination of the P100 component and may yield additional VEP descriptors.

P07

Intensity-dependent impacts of exercise on cognitive functions in multiple sclerosis - preliminary results of a randomized controlled trial

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Background. The influences of exercising on cytokine response, cardiorespiratory and cognitive functions are important aspects of rehabilitation in persons with multiple sclerosis (pwMS) but have not been systematically investigated. Recent data study show positive connections between elevated neurotrophin concentrations, induction of neuroplasticity, recovery of the motor and cognitive functions and the applied training intensities in pwMS.

Objective. This study determines the immune response of neurotrophic factor BDNF and cognitive functions to 3-weeks of endurance training conducted a cycle ergometer and progressive resistance training. The main objectives are to (a) investigate whether intensive exercise has similar effects on growth factor BDNF, cognitive functions and cardiorespiratory fitness than normal exercise and to (b) examine which modality is more effective at affecting immune and cognitive functions in pwMS.

Methods. A randomized controlled clinical trial is conducted in 80 MS patients (Expanded Disability Status Scale range 1.0–6.5) however here only results of 20 pwMS are presented. Resting serum levels of brain-derived neurotrophic factor (BDNF) and the concentrations in response to cardiopulmonary exercise test (CPET), fatigue and cardiorespiratory values were determined at entry and discharge. Cognitive functions are assessed with the German version of the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) and additionally, the German version of the Trail-Making-Pencil-Test versions A/B (TMT-A/B) were used. Cognitive assessments were performed at baseline (t1) and repeated at t2. Participants were allocated into a group that performed intensive (IT) or normal training (NT). Groups differ by means of frequency and the intensity of the training session. IT has less sessions that are attuned (3 active versus 3 passive). Daily training session sum-up to six in the IT and eight in the NT. NT is the conventional training performed in the Valens clinic.

Results. BDNF show significant differences between groups over the training intervention. Within NT BDNF resting and post-CPET concentrations ($p<0.05$) show a significant increase after the training intervention. Short-term effects on BDNF (CEPT) tended to increase at the start and significantly thereafter ($p<0.05$). No changes occurred in the NT group. Cognitive functions show time effects on the BVMT-R of the BICAMS over the training intervention Cardiorespiratory fitness improved significantly over time within both groups.

Conclusion. This study indicates that intensive exercise activates BDNF regulation and can be an effective training modality in pwMS.

P08

Time to relapse and disability progression in a long-term cohort of people with clinically isolated syndrome and relapse-onset multiple sclerosis treated with disease-modifying drugs: a prospective nationwide survey in Switzerland

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Background. The efficacy of disease modifying drugs (DMDs) to prevent relapses in relapsing-remitting multiple sclerosis (RRMS) has been shown in numerous Phase-III trials. However, the long-term effect on disability progression is still a matter of debate.

Aim. To determine time to relapse and disability progression in a large long-term MS cohort treated with DMDs.

Methods. Analysis of data from the Swiss Federation for Common Tasks of Health Insurances (SVK) that includes standardised annual information on diagnosis, disease onset, relapses and neurological status assessed using the Expanded Disability Status Scale (EDSS) of about 80% of all MS patients treated with DMDs in Switzerland. The case record forms provided by the treating neurologists were reviewed for completeness and internal plausibility and queries about missing or inconsistent data were issued by the SVK under the supervision of an independent physician. EDSS progression was defined as ≥1 step if EDSS was ≤5.0 and ≥0.5 if EDSS was ≥5.5 confirmed at two consecutive annual evaluations. Patients who switched or discontinued treatment before confirmation of the EDSS change were censored. Hazard ratios were propensity-score adjusted for clinically relevant baseline characteristics including age, gender, disease duration, disease subtype, EDSS at treatment start and time of DMD introduction.

Results. From 1995 to 2010, 8044 patients were included in this study: 472 clinically isolated syndrome, 6832 RRMS, 740 secondary-progressive MS (SPMS); mean age 39.6±11.3, disease duration 7.15±8.01 years, annualised relapse rate 0.91±0.66 [based on the two antecedent years], median EDSS at treatment start 2.5 (range 0-8), mean time of follow-up 4.4±3.94 years. In the year prior to treatment start, 16.6% of the patients were relapse-free. This proportion increased after one year of treatment to 60.6% similar across the treatment groups. After 10 years on DMD treatment, 15.1% of the patients had still relapses. Median time to confirmed EDSS progression was 7.92 years (interquartile range [IQR] 7.68-8.74) in RRMS and 4.96 years (IQR 4.33-5.97) in SPMS calculated from treatment start. In a propensity-score adjusted analysis, both the median time to relapse and confirmed EDSS progression was similar across the treatment groups.

Conclusions. In this comprehensive and large long-term MS cohort, time to relapse and confirmed EDSS progression was similar across interferon-beta products and glatiramer acetate.

P09

Correlation of Nine Hole Peg Test and MS related corticospinal tract damage assessed by the triple stimulation technique

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Introduction. Demyelinating lesions in Multiple sclerosis (MS) can affect the corticospinal tract and lead to motor impairment of the upper extremity (UE). UE function is usually evaluated using clinical assessments such as the Nine Hole Peg Test (9HPT). Impairment of the corticospinal tract can be electrophysiologically assessed using the triple stimulation technique (TST).

Aim. We aimed to assess the relationship between performance on the 9HPT and corticospinal tract damage as assessed by TST.

Methods. We performed 9HPT and TST in patients with MS on both arms. Pearson correlation (r) was used to investigate the relationship between 9HPT and TST. Correlation was assessed for left and right arm separately.

Results. We examined 45 MS patients (29 RRMS, 14 SPMS, 2 PPMS, Median EDDS 3.5 (1.5 – 7.0), Mean age 49.0 years (23-72). Results of the 9HPT ranged from 15.5 sec – 107 sec (left arm) and from 14.55 sec - 55.7 sec (right arm). We found a correlation between 9HPT and central motor conduction time (CMCT) for both arms (Pearson correlation left: $r=0.53$, $p<0.001$; right: $r=0.52$, $p<0.001$). The proportion of activated motor units and performance in the 9HPT was negatively correlated: (Left: $r=-0.38$, $p=0.008$; Right: $r=-0.52$, $p<0.001$).

Conclusion. In MS patients performance on the 9HPT is related to corticospinal tract damage as reflected by prolonged CMCT and a reduced number of activated motoneurons. The lower correlation between performance in the 9HPT and proportion of activated motor units might be related to the patients handedness as 93% of the patients were right hander. Performance of the non-dominant hand in the 9HPT is worse in healthy people and may have lower susceptibility for loss of neurons of the corticospinal tract.

P10

Spinal cord gray matter atrophy in early multiple sclerosis

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Aims. Spinal cord gray matter (GM) atrophy has been recently described in vivo in patients with long-standing Multiple Sclerosis (MS) and has been shown to correlate with disability and disease type. The goal of this study was to assess whether spinal cord GM atrophy starts early in the disease and whether it equally affects the cervical and thoracic cord.

Methods. 40 patients at an early stage of MS (mean age 36.5 years, 29 women, mean disease duration from first symptom onset: 1.3 years (range 0-3.7 years)) and 20 age and sex matched healthy controls were scanned at 3T. Axial 2D-phase sensitive inversion recovery MR images were acquired at the intervertebral disc levels C2/C3 and T9/10. Total cord areas (TCA) were segmented semi-automatically, spinal cord GM areas were segmented manually, and spinal cord white matter (WM) areas were calculated as their difference. Differences in areas between patients and controls were assessed with age and sex as covariates using multivariable regression analysis.

Results. In the cervical and thoracic spinal cord MS patients had significantly smaller spinal cord GM areas than age and sex matched controls (Coefficient of variation (COV) 7%, $p<0.001$ at C2/C3 and 7%, $p=0.04$ at T9/T10), but had no significant difference in either the spinal cord WM area or TCA.

Conclusions. These observations demonstrate that spinal cord GM atrophy can be detected already at an early stage of MS, in the absence of WM atrophy, and equally affects both the cervical and thoracic cord. Longitudinal, prospective studies are necessary to clarify the role of cord GM changes in monitoring and predicting MS disability and progression.

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P11

The Swiss Multiple Sclerosis Registry (SMSR): a citizen science platform for MS research

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Aims. Multiple Sclerosis (MS) disease management requires active involvement of persons with MS (PwMS), families, and caregivers. It also depends on a well-organized interplay of health care professionals. However, in Switzerland there is a widely recognized lack of information and high quality evidence on disease epidemiology, long-term efficacy and safety of disease-modifying drugs, access to and uptake of MS treatments and care, or needs and preferences of PwMS.

Methods. The Swiss Multiple Sclerosis Registry (SMSR) is based on an initiative of the Swiss MS Society. It is a patient-centered, nationwide, longitudinal study that will be open to all adult PwMS living in Switzerland. Enrollment will start in early 2016. The SMSR takes a citizen science approach: it attempts to involve PwMS not only as study subjects but also as MS experts, whose opinions and experiences are valued. Other notable features of the SMSR are the flexible study design that allows participation at different commitment levels (i.e. from one-time surveys to longitudinal data collections), the ownership of data by registry participants (who will have access to their own data), and the possibility to include participant-provided, unstructured information such as medical reports. Main scientific objectives of the registry entail: 1. to estimate the prevalence of MS in Switzerland and to monitor epidemiologic trends over time, 2. to estimate the burden of MS for PwMS and families or proxies, and 3. to establish a flexible infrastructure and a network that enables and facilitates interdisciplinary research with all interested partners. Participation will be possible by paper questionnaire or by data entry into a newly designed online platform. This platform will also offer MS disease management tools for PwMS and physicians (e.g. life charts). Surveys will cover a wide range of topics on disease history, circumstances of living, mental health, MS

treatment (drug and non-drug), or coping with MS. In addition to patient-reported survey outcomes, the SMSR will further collect clinical data through medical record abstraction. Conclusions: The SMSR will be a unique addition to the Swiss and the European MS research landscape for its innovative design and strong involvement of PwMS and their relatives in data collection and research agenda design. Thereby, it will complement other ongoing longitudinal Swiss research efforts, with which the SMSR will seek close collaborations and interaction

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Members of the Swiss Multiple Sclerosis Registry. Anderseck B, Beer K, Calabrese P, du Pasquier R, Engelhardt B, Gobbi C, Jolley N, Kägi S, Kesselring J (President), Kuhle J (Chair of Clinical and Laboratory Research Committee), Kunszt P, Kurmann R, Lotter C, Luyckx K, Merkler D, Nedeltchev K, Puhan M (Principal Investigator), Rapold I, Schippling S, Schluep M, Vaney C (Chair of Patient and Population Research Committee), von Wyl V (Chair of IT and Data Committee).

P12

The French version of the Multiple Sclerosis Questionnaire for Physiotherapists: a reliable and valid method for the evaluation of the treatment of persons with MS

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Objectives. To improve the quality of physiotherapeutic intervention of persons with MS, the Specialized Group Physiotherapy in MS of Physioswiss has developed the Multiple Sclerosis Questionnaire for Physiotherapists(MSQPT), a disease-specific self-rating questionnaire. This study evaluates the psychometric properties of the French version of the MSQPT.

Method. The German MSQPT was translated using the same procedure as the transcultural adaptation of the SF-36 into different languages. The quality of the translation, the reliability and validity of the French version and the acceptance by physiotherapists were evaluated. The survey was conducted in the French speaking part of Switzerland. Patients (pretest n=5, validity testing n=31, test-retest reliability n=16) were recruited in private practices, hospitals and rehabilitation centers. The intervention used the MSQPT and the SF-36. Furthermore the self-administered EDSS score was determined. The treating physiotherapist filled out a questionnaire to estimate the acceptance of the MSQPT.

Results. The rating of clearness, everyday speech and conformity of concept of the translation was high for most items except for one. It was excluded from the French MSQPT. The final MSQPT did not show any other problem in the pretest and was used for the validation survey. The quality of the validation data was high. The survey denoted few missing data(MSQPT 0.64%, test-retest 0.09%, SF-36 0.77%). The survey is not representative for the Swiss MS population (48% woman). Validity: The criterion validity between the MSQPT and the SF-36 was high (activity r=0.85, participation associated factors versus social functions, vitality and well-being r=0.47-0.74, pain r=0.64). Reliability: The French MSQPT has an overall intern consistency of 0.84 (Crohnbach α). The two main groups have a Crohnbach α of 0.82 and 0.87. Many items have a high test-retest reliability. The activity group and the total score have a very high reliability(r=0.93 resp. 0.95). The participation group has a low reliability score (r=0.66). The MSQPT has a very high acceptance and was rated as simple, comprehensible, efficient, not time-consuming and very useful.

Conclusion. The French MSQPT is a well translated questionnaire of high quality. Its psychometric properties are good and comparable to the original German version. The results suggest that the French MSQPT can be used in the evaluation of the physiotherapeutic treatment of MS patients.

P13

Patient Reported Questionnaire in MS Rehabilitation: Testing Responsiveness and Minimal Important Difference of the French Version of the Multiple Sclerosis Questionnaire for Physiotherapists

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Objectives. The Multiple Sclerosis Questionnaire for Physiotherapists (MSQPT) is a German PRO questionnaire for the evaluation of the rehabilitation of persons with MS. The focus of this study is the evaluation of the responsiveness and Minimal Important Difference of the French version of the MSQPT.

Method. We used a combined anchor and distribution based approach with multiple anchors and multiple transition questions. The intervention (n=31) included the French MSQPT, the French Version of the Hamburg Quality of Life Questionnaire in Multiple Sclerosis (HAQUAMS), the self-administered EDSS, 6 Meter Timed Walking Test (6TWT), Berg Balance Scale(BBS) and the 6 Minute Walking Test (6MWT). Responsiveness was evaluated using ES, SRM and Modified SRM. The distribution based estimates 0.33, 0.5 SD, SEM, MDC90 and MDC95 were calculated to evaluate the MID that were established for the German MSQPT. The specificity of the MID and the correlation between the physical tests and the items and groups of the MSQPT were determined. The relative efficiency between the French MSQPT and HAQUAMS was estimated.

Results. The main ES for deterioration lay between 0.41 and 0.93 and for improvement between 0.42 and 1.23. The SRM are generally higher than the ES (deterioration 0.89 to 2.14, improvement 1.08 to 2.14). Main Modified SRM range 0.03 to 0.31 and are acceptable. The specificity of the MID range from 0.25 to 0.83. Comparing responsiveness of the German and the French MSQPT, the data is not unambiguous, but in general the differences between estimates are small. The correlations between BBS and 6 MWT and the items and groups of the MSQPT are reasonable to high (0.51 to 0.74). The French MSQPT seems to be more efficient than the French HAQUAMS in detecting improvement but less in finding deterioration.

Conclusion. Due to the small sample size (n=31) the significance of this survey is limited. The available evidence indicate that the French MSQPT is a responsive PRO questionnaire, with similar psychometric characteristics as the original German MSQPT. The French MSQPT has adequate MID that may be used as thresholds for change in the physiotherapeutic treatment of persons with MS.

P14

The High-Dimensional TH Cell Landscape in Multiple Sclerosis

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Multiple sclerosis (MS) is a common autoimmune disease with an underlying T cell-based etiology. Particularly T helper (TH) cell differentiation and cytokine production are critical mechanisms in the pathogenesis of MS. After the initial discovery of distinct TH1 and TH2 cell subsets that produce interferon-γ and IL-4 respectively, numerous additional polarization patterns (TH9, TH17, TH22, TFH) have been proposed. The exact identity of the TH cell polarization pattern and the responsible cytokines underlying MS pathology have been the focus of intense research for several decades, however these questions still remain unresolved.

The aim of this study is to reveal the breadth of cytokine production profiles, chemokine receptor and activation marker expression of the enitre TH cell landscape in MS. More specifically, we employ the recently developed mass cytometry technology for which we developed a 42 parameter panel to determine the exact identity of the MS-associated TH cell polarization pattern. To analyse those high-dimensional cytokine expression patterns in an unbiased manner, we apply unsupervised dimensionality reduction algorithms (t-SNE) followed by automatic classification (ACCENSE). In a next step, we currently apply this approach to peripheral leukocytes from healthy individuals in order to obtain a composite picture of the degree of cytokine co-production by TH cells in an unprecedentedly high-dimensional space. Finally, we plan to compare those TH cell landscapes from control and MS patients in various stages of disease. Therefore, we will employ algorithms performing automated identification of stratifying cellular subpopulations (citrus).

Together, this analysis could thus indicate the chemokine receptor and cytokine expression profile of the true pathogenic T cell thus resolve longstanding controversies about the relative importance of different cytokines in MS.

P15

Claudin 3-deficient C57BL/6 mice display intact brain barriers

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During neurological disorders such as multiple sclerosis (MS) or its animal correlate experimental autoimmune encephalomyelitis (EAE), focal loss of blood-brain barrier (BBB) integrity is observed and associated with the formation of inflammatory lesions as visualized by gadolinium-enhanced magnetic resonance imaging (MRI). Claudin-3 is localized to tight junctions (TJs) of the endothelial blood-brain barrier (BBB) and the epithelial blood-cerebrospinal fluid barrier (BCSFB). A specific contribution of claudin-3 in BBB integrity has been suggested by its selective loss in microvessels surrounded by inflammatory infiltrates in EAE. Additionally, claudin-3-/- mice on a mixed genetic background have recently been shown to develop aggravated EAE due to increased leakiness of the BCSFB. This prompted us to study EAE in the homogenous genetic background of C57BL/6 mice. To this end we have generated claudin-3-/- mice and after their backcrossing for 10 generations on the C57BL/6 background studied EAE pathogenesis. To our surprise we did not observe any significant difference in disease development in Claudin-3-/- C57BL/6 mice when compared to their WT littermates.

These studies were accompanied by investigations on the barrier properties of the BBB and the BCSFB. Measuring transelectrical resistance and permeablity of small and large molecular tracers across the BBB and the BCSFB in vitro and in vivo revealed no difference in barrier properties of WT or claudin-3-/- C57BL/6 mice. Taken together, our results demonstrate that absence of claudin-3 in C57BL/6 mice does not impair brain barrier properties during health and autoimmune neuroinflammation.

P17

Reprogramming inflammatory monocytes by antibodies to colony-stimulating factor 1 receptor prevents sickness behavior in inflammatory diseases

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Sickness behavior syndrome (SBS) as characterized by fatigue and depression impairs quality of life in patients with autoimmune and infectious diseases. Inflammation in mice induced by agonistic CD40 antibodies leads to SBS, which at sites of inflammation is associated with influx of Ly6Chigh monocytes and loss of the F4/80high macrophages and Ly6Clow monocytes. The aim of the study presented here is to test whether neutralization of colony-stimulating factor 1 receptor (CSF1R) protects from CD40 mediated SBS.

Results. Antibodies to CSF1R depleted the subset of CD11b+Ly6CnegCD115+ macrophages and CD11b+Ly6C+F4/80high macrophages, but polarized CD11b+Ly6Chi inflammatory monocytes to a mixed phenotype with increased TNF and IL-10. This immune phenotype was associated with protection of mice from SBS.

Discussion. The increased expression of IL-10 in CSF1R antibody treated mice overrides the negative effects of pro-inflammatory cytokines on behavior and body weight. These data provide a major conceptual advance in understanding the molecular and cellular events leading to SBS.

P18

Sterile inflammation in MS: Induction of cytokine production in human monocytes by T cell surface bioactive lipids

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Aims. Imbalance in cytokine homeostasis plays an important part in the pathogenesis of MS. Stimulated T cells display pathological effects through direct cellular contact with monocytes/macrophages, inducing a massive up-regulation of interleukin-1β (IL-1β) and tumor-necrosis factor (TNF) in the latter cells. This suggests the presence of activating factors at the surface of stimulated T cells. Our recent results demonstrated the lipidic nature of these factors. The aim of our work is to characterize T cell bioactive lipids referred to as SAFT (surface activating factors on stimulated T cells).

Methods. Total lipids extracted from membranes of stimulated and unstimulated HUT-78 cells were subjected to serial solid phase extraction on 3 different types of cartridge. Lipid fraction activity was assessed on isolated human monocytes by measuring induction of IL-1β and its inhibitor IL-1Ra. Fractions were analyzed by high resolution mass spectrometry (MassSpec) on QExactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer. Lipid composition of active fractions generated from membranes of stimulated HUT-78 cells was compared to that of fraction obtained from unstimulated HUT-78 cells.

Results. Preliminary results ruled out the participation of acyl sphingolipid species, sterols, sterol esters, ether lipids, and neutral fatty acids in SAFT activity. The primary MassSpec analysis showed that 1421 m/z were increased more than 2 times in stimulated as compared to unstimulated HUT-78 cells. The comparison of lipids enhanced in active fractions of stimulated HUT-78 cells and absent in inactive fractions as well as in fractions isolated from unstimulated HUT-78 cells resulted in 6 putative lipid m/z which might display SAFT activity. None of the latter corresponded to a lipid in the LIPIDMAPS data base, i.e. the largest available lipid data base (www.lipidmaps.org). However, lipid data bases are currently under construction and thus do not contain all possible natural lipids. To circumvent this hurdle, the 6 m/z identified are currently subjected to fragmentation to obtain better insights into their structure.

Conclusions. 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.

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P19

Comparison of subjective and objective adherence in patients with multiple sclerosis using RebiSmart™

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Aims. The RebiSmart autoinjector delivering subcutaneous (sc) interferon (IFN) β-1a records objective adherence data and enables patients with multiple sclerosis (MS) to overcome factors leading to poor adherence. The aim of this study was to compare objectively recorded dosing history using RebiSmart with subjectively patient-reported adherence, and identify potential factors impacting therapy adherence in patients using RebiSmart.

Methods. A Swiss, multicenter, observational practice survey of MS patients treated with sc IFN β-1a 44/22 µg using RebiSmart for ≥9 months. Primary endpoint was the difference between objective adherence measured using RebiSmart and subjective adherence captured by a patient questionnaire (one-way analysis of variance). Secondary endpoints: i) difference between objective adherence 9 months before baseline (retrospective) and 6 months after baseline (prospective, Wilcoxon matched pairs test); ii) questionnaire-based identification of potential dependent variables in patients with low (<90%), medium (90–99.99%), and high (>99.99%) objective adherence (ordinal regression). Self-reported adherence and non-adherence were defined as missing 0 and ≥1 injections, respectively, during 9 months preceding baseline. Data are mean±SD.

Results. 53 of 56 patients (age 48.2±12.1 years; 22.6% male) completed the study. Objective adherence with RebiSmart in the self-reported compliant (n=33) and non-compliant groups (n=20) was 97.4±0.4% and 78.0±7.6%, respectively (p<0.001). Retrospective and prospective adherence measured with RebiSmart was 90.1±3.9% and 90.7±3.5%, respectively (p=0.75). Objective adherence was significantly associated with increasing age (low=42.3±12.0, medium=47.6±11.5, high=53.1±11.0; p=0.006) and Expanded Disability Status Scale (low=1.6±0.9, medium=2.2±1.4, high=2.7±1.2; p=0.006), neurologists' estimations of adherence (low=8.5±2.3, medium=8.9±1.2, high=9.6±0.7; p=0.023), the importance of simplicity (low=8.3±1.5, medium=9.1±1.7, high=9.7±0.9; p=0.01), ease of storage (low=6.9±2.6, medium=7.3±2.8, high=8.7±1.7; p=0.032), and good information about RebiSmart features (low=9.5±0.7, medium=9.7±0.6, high=10±0.0; p=0.009).

Conclusions. MS patients in Switzerland using sc IFN β-1a via RebiSmart had very high real-life treatment adherence. Objectively measured adherence was associated with both patient self-reported and neurologists' estimated adherence. Older and more disabled patients tended to be more adherent to treatment.

P20

The Role of the Junctional Adhesion Molecule (JAM)-B in the Pathogenesis of Experimental Autoimmune Encephalomyelitis

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Under physiological conditions the endothelial blood-brain barrier (BBB) maintains homeostasis in the central nervous system (CNS) by restricting the uncontrolled diffusion of molecules or trafficking of immune cells into the CNS. Paracellular diffusion of molecules across the BBB is inhibited by complex and continuous tight junctions (TJ) between the endothelial cells. Besides the transmembrane proteins occludin, claudin-3 and -5, the junctional adhesion molecules (JAM-A, JAM-B and JAM-C) have been shown to be localized to BBB TJs.

During multiple sclerosis (MS) focal loss of BBB integrity is a hallmark of disease pathogenesis as visualized by gadolinium-enhanced magnetic resonance imaging. In the animal model of MS, experimental autoimmune encephalomyelitis (EAE) autoaggressive T cells breach the BBB and cause inflammation, edema and demyelination, which set the stage for the development of the clinical disease. Targeting T cell trafficking across the BBB by blocking VLA-4 with the humanized antibody natalizumab has proven beneficial for the treatment of MS, however, comes with the risk of progressive multifocal leukoencephalitopathy (PML). It is thus mandatory to further investigate BBB proteins allowing to selectively block the CNS entry of autoreactive immune cells.

Based on the genuine role of VLA-4 in T cell trafficking into the CNS during EAE and MS, the observation that endothelial JAM-B can bind VLA-4 on human T cells is intriguing. Using novel transgenic mouse models with a constitutive lack of JAM-B we investigated its function in maintaining TJ integrity and how it influences the migration of different immune cell subsets across the BBB during EAE. We found that absence of JAM-B does not impair BBB integrity or influence T cell migration across the BBB in vitro.

Interestingly, JAM-B-/- C57BL/6 mice showed ameliorated active EAE, while JAM-B-/- SJL/J mice developed EAE with the same severity as their wild-type littermates. This might be due to the significant differences in the VLA-4 expression on encephalitogenic T cells observed by us between the two mouse strains. Histological analysis of brain and spinal cord sections of JAM-B-/- C57BL/6 mice afflicted with EAE showed reduced parenchymal infiltration of CD45+ inflammatory cells accompanied by their increased accumulation in leptomeningeal and perivascular spaces when compared to wild-type littermates. At this stage our data point to a role of JAM-B in EAE pathogenesis in the C57BL/6 mouse.

P21

Assessing Short and Graphically the Mobility in MS and Other Neurological Disease with the new iPhone App SaGAS 10

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Background. SaGAS 10 is a new iPhone app developed as an alternative to the MS Functional Composite (MSFC) and as a complement to the EDSS for the moderately disabled MS patients between EDSS 5.0 and 7.0. Assuming that this tool could also be used for other neurological diseases where walking and hand function is impaired, we set out to examine the validity and the responsiveness of SaGAS 10 in neurological patients attending a rehabilitation facility.

Methods. 646 consecutive patients with different neurological diseases (MS 296, stroke 152, Parkinson 21, neuromuscular disorders 42, trauma 42, others 93) were assessed at the beginning and at the end of their rehabilitation stay using the FIM (Functional Independence Measure), the RMI (Rivermead Mobility Index, the 2-minute timed walking distance at maximum speed (2MWD) and the 3 measures composing SaGAS 10 (the 25 feet timed walk at fast speed with a flying start (T25FW) and the nine-hole peg test (9-HPT) for each hand separately). Construct validity was assessed with correlations between FIM, RMI and the SaGAS 10, where correlations above 0.7 were hypothesized. Responsiveness was assessed by a receiver operating characteristic curves (ROCs) analyses comparing changes in SaGAS with minimal clinically important changes in the RMI. An area under the curve value (AUC) of at least 0.7 was considered as appropriate.

Results. The correlation of the SaGAS 10 with the Rivermead Mobility Index is above 0.7 in all of the neurological diagnostic groups; the highest correlation coefficient was found in patients with stroke: 0.75 (95% CI 0.63 to 0.83). The correlation of the SaGAS 10 with the FIM was over 0.7 for stroke and MS. The responsiveness was acceptable with AUCs of 0.71(95% CI 0.59 to 0.83) for stroke and values over 0.7 for all groups, with the exception of MS (AUC 0.61, 95% CI 0.46 to 0.76). The effect-sizes were moderate to high, especially for stroke with Cohen's d values of 0.48 for the whole group and higher values for those walking slower (ES 0.61 for under 1.04 m/s and ES 0.72 for speed under 0.96 m/s).

Conclusions. These results indicate that SaGAS 10 is valid and sensitive to changes over time and that it could be a useful measure not only for patients with MS, but also for patients with other neurological diseases such as after stroke.