to 59,7% of the results on the gait test can be explained with all the variables included. Of the included variables included the time spent in the final part of stance phase shows the greatest amount of association, which alone explains 57,3% of the results. **Conclusion:** Push-off appears highly relevant for the ability to walk long distances on a gait test. The use of wearable technology sensors offers the clinician a tool to assess and evaluate changes related to gait. This can be used in clinical practice without any negative impacts on established routines.

Disclosure
Thomas Klyve: Nothing to disclose

Scientific Session 8: Immune cell trafficking into the CNS

O078
Immune cell trafficking across brain barriers

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Multiple sclerosis (MS) is the prototypical idiopathic neuroinflammatory disorder. MS affects young adults and children, and is clinically characterized by bouts of focal inflammatory activity in the brain and spinal cord, leading to unpredictable and temporary clinical episodes of dysfunction, such as optic neuritis (causing vision loss) or transverse myelitis (leading to numbness and or limb paralysis). Experimental allergic encephalomyelitis, when induced in mice, remains a good, although very imperfect, model of MS. In both MS and EAE, the disease evolves as a relapsing remitting disease, followed by irreversible progression of handicap. Pathological analyses of human brains of patients affected by MS, and of CNS from animals affected by EAE, provided insight on the immunopathology of these diseases. We now know that these CNS-targeted disorders involves CD4 and CD8 lymphocytes, monocytes-macrophages-dendritic cells, microglia and astrocytes which have different and sometimes divergent or opposing effects on the various phases of the disease. We also know that oligodendrocytes and neurons are the main target of the immune attack, and that endothelial cells also contribute to the recruitment of immune cells into the CNS.
Using recent technological advances in Flow cytometry, scRNAseq, cell biology, microscopy and system biology allowed the field to provide a thorough characterization of the role of each cell type in neuroinflammation, both in human MS and in EAE, including molecules involved in trans-endothelial migration (CAMs, chemokines and chemokine receptors). The lecture will present some novel data on this topic, focusing on endothelial molecules involved in diapedesis of immune cells across vascular brain structures.

Disclosure
Nothing to disclose

O079
T cells are poised to invade the CNS through a distinct transcriptional cytotoxic program in MS

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Introduction: In MS patients, pathogenic $CD4^+$ T cells cross the blood-brain barrier, also allowing other immune populations, including $CD8^+$ T cells, to infiltrate the brain. How transcription factors known to control the cytotoxic T-cell program are involved in this process is underexplored.
Objectives: To assess whether changes in the cytotoxic T-cell program explain the propensity of $CD4^+$ and $CD8^+$ subsets to enter the MS brain.
Aims: By delineating which intrinsic factors underlie brain homing of human T cells, we aim to better understand the cause of MS.
Methods: Expression patterns of RUNX3, EOMES and T-bet as key determinants of the T-cell effector program were examined and associated with cytotoxic and brain-homing features of pathogenic $CD4^+$ (Th17.1) and $CD8^+$ ($CD20^{dim}$) T cells by FACS. We used blood and CSF samples as well as brain tissues from different MS cohorts. The brain-homing capacity was verified by studying blood of natalizumab-treated MS patients and by performing in vitro BBB transmigration assays.
Results: Within both $CD4^+$ and $CD8^+$ T cells, the frequency of RUNX3-expressing memory populations with cytotoxic potential ($CD107a^+$) was reduced in the blood of 18 treatment-naïve early MS patients versus 8 matched healthy controls ($p<0.001$ and $p<0.01$, respectively). This decline was restored after natalizumab treatment (n=8). Similar results were obtained for EOMES, which was mainly expressed by $CD8^+$ T cells. In RUNX3-expressing cells, we found an additional loss in T-bet, which corresponded to the presence of MS risk SNP rs6672420 (*RUNX3*). In contrast to $RUNX3^+EOMES^-T\text{-}bet^+$ (granzyme B^+) cells, $RUNX3^+EOMES^+T\text{-}bet^-$ cells displayed high levels of CCR5 and granzyme K, which were enriched in $CD20^{dim}$ and $CD69^+$ subsets. This was most prominent in MS CSF, where Th17.1 cells predominated the memory T-cell compartment compared to paired blood (n=15) and control CSF (n=8). Of all $CD4^+$ T-cell subsets analyzed, Th17.1 had the highest proportion of $RUNX3^+EOMES^+T\text{-}bet^-$ cells and produced granzyme K, especially after crossing the BBB in vitro. In post-mortem MS brain tissue (n=8), T-bet was reexpressed and EOMES was downregulated in predominating $CD20^{dim}(CD69^+)$ $CD8^+$ T cells, which was accompanied by granzyme B and K coproduction.
Conclusions: This work reveals that in MS patients, coexpression of RUNX3 and EOMES, but not T-bet, defines $CD4^+$ and

$CD8^+$ T-cell subsets with a cytotoxic profile that likely contributes to their preferential recruitment to the CNS.

Disclosure
J.S. received lecture and/or consultancy fees from Biogen, Merck, Novartis and Sanofi-Genzyme. M.M.v.L. received research support from EMD Serono, GSK and Idorsia Pharmaceuticals Ltd.

O080
Blood-borne immune cells in the central nervous system: high-dimensional single cell characterization of regional heterogeneity in multiple sclerosis

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Background: Characterization of immune compartments in different regions of the central nervous system (CNS) is essential to understand brain immunity and its dysregulation. However, in multiple sclerosis (MS), although brain microglia regional heterogeneity has been well studied, the CNS regional heterogeneity of blood-borne immune cells remain poorly explored.
Objectives: 1) To characterize the regional heterogeneity of blood-borne immune cells in the CNS of patients with MS at a high-dimensional single cell level.
2) To define the population of blood-borne immune cells within the CNS that are dysregulated in MS pathology.
Methods: The pia, choroid plexus, corpus callosum, cerebral cortex, cerebral white matter and cerebellum from fresh brain, as well as post-mortem peripheral blood was obtained from two MS patients and four patients with other neurological diseases (OND) including amyotrophic lateral sclerosis (n=3) and hereditary spastic paraplegia (n=1). Tissue was processed to obtain a single cell suspension of immune cells, and cells were stained with 21-color flow cytometry panel developed to extensively characterize different immune cells and their phenotype. To extensively study the regional immune cell heterogeneity, unsupervised clustering and data vizualization was performed with FlowSOM, UMAP, and Multiscale PHATE algorithms. Cells and clusters identified in MS patients were then compared with those observed in OND patients to characterize the MS-specific immune signature and heterogeneity across CNS regions.
Results: After excluding doublets, dead cells, and microglia we have successfully retrieved more than 400 000 immune cells from 61 brain regions (230 686 cells from MS, 185 976 cells from OND). In a preliminary analysis of a subset of the dataset including pia and post-mortem peripheral immune cells, we have shown that we can characterize immune cell populations and their subsets in both compartiments (T cells, B cells, NK cells and dendritic cells). Moreover, we have demonstrated that immune cells residing within the pia show a distinct immunophenotype when compared to peripheral immune cells.
Conclusions: Our high-dimensional single cell characterization of the human brain will allow us to define the regional heterogeneity in CNS of patients with MS and other neurological disorders. Understanding the distinct immune signatures across brain regions in the MS brain will provide important insights into the physiopathology of the disease.

Disclosure:
The authors have nothing to disclose.

O081
Osteopontin as brain T_{RM}-cellhallmark relates to compartmentalization and MS-pathology

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Introduction: Perivascular space-compartmented tissue-resident memory T (T_{RM}) cells are physiological residents of the central nervous system (CNS), presumably to control neurotropic virus reactivation. We recently showed that MS normal-appearing white matter (NAWM) is populated by increased numbers of perivascular space-restricted $CD8^+$ T_{RM} cells. In active white matter lesions, $CD8^+$ T cells are even more abundant and have an increased capacity to leave the perivascular space and invade the parenchyma.
Objectives: Currently, we investigate and validate transcriptomic changes in MS and non-MS brain T_{RM} cells.
Aims: Through this, we aim to find putative targets in brain T_{RM} cells that can ameliorate progressive MS.
Methods: Of n=11 non-MS post-mortem brain donors, we isolated $CD8^+$ and $CD4^+$ effector memory and effector memory re-expressing CD45RA T cells from blood and $CD8^+$ and $CD4^+$ T_{RM} cells from white and grey matter. Additionally, these cells were sorted from paired normal-appearing white and grey matter and from white and grey matter lesions of n=6 MS brain donors. Subsequently, isolated cells were sequenced. Validation experiments were conducted through flow-cytometry and immunohistochemistry.
Results: Bulk RNA sequencing of these T cells revealed a core T_{RM}-cell signature. Hallmark upregulated transcripts in brain T_{RM} cells were *SPP1* (osteopontin) and *MS4A1* (CD20). Osteopontin (OPN) depositions were noted in close contact with perivascular