Diversity of microglia

Their contribution to multiple sclerosis lesion formation

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Chapter 10

Summary

The scope of this thesis is to identify changes in microglia reflecting neuropathological changes in the human brain (part 1) and to study the contribution of microglia to MS lesion initiation (part 2) and development (part 3).

The effect of ante- and post-mortem variables on microglia characteristics isolated from post-mortem human brain tissue is assessed in **Chapter 2**. We optimized a protocol to rapidly isolate human microglia within 4 hours with an average yield of 450.000 (white matter) and 150.000 (grey matter) viable cells per gram of post-mortem brain tissue, with a short post-mortem delay (on average 6 hours). We compared over 100 microglia isolations to assess the impact of clinical and autopsy parameters on cell viability and microglial CD45/CD11b expression. Except for pH of the cerebrospinal fluid (CSF), reflecting brain acidity, age, post-mortem delay, total time until isolation and donor diagnosis did not correlate with microglia yield. Donor parameters (other than neurological diagnosis), including age, brain acidity, total time until isolation and post-mortem delay had no effect on microglial CD45/CD11b expression. Furthermore, we demonstrated that microglia can be distinguished from autologous choroid plexus macrophages based on size, granularity and CD45/CD11b expression using flow cytometry. Finally, we showed that microglial gene expression is drastically changed within hours upon culture conditions and provided evidence that microglia can be cryostored for brain bank purposes while retaining high RNA quality and microglial phenotyping is still possible, allowing researchers to collect cells for later analyses. Combined, we showed that human microglia isolated from post-mortem brain tissue retain their viability and phenotype, allowing to reliably relate microglial changes, such as CD45 expression, to the neuropathological status of for instance MS donors.

Chapter 3 describes cell isolation protocols and analytic techniques, such as multi-omics, flow cytometry and functional assays, to phenotype primary human glial cells isolated from post-mortem brain tissue. Main focus is on previously published isolation techniques to obtain glial cell populations from human brain tissue with short post mortem delay (on average 6 hours), including the rapid isolation protocol that we developed in our group to obtain viable microglial cells within 4 hours from post-mortem tissue. Furthermore, downstream applications to profile primary human glial cells in order to identify changes related to neurological or psychiatric diseases are reviewed.

BAI1 is a phagocytic receptor that mediates the uptake of apoptotic cells and is involved in neural development and angiogenesis. Several studies reported on the role of this receptor in primary cells and cell lines of monocyte and/or macrophage origin. In **Chapter 4**, we tested various data sets of gene expression in monocytes, monocyte-derived macrophages and tissue macrophages, including microglia, from human, mouse, and zebrafish, but failed to find evidence for expression of BAI1 at mRNA and protein level. This brief report does not question the role of BAI1 as phagocytic receptor, but challenges the expression of BAI1 on monocytes and macrophages demonstrated by previous studies.

In **Chapter 5**, we identified the transcriptional profile of human microglia, which were isolated from post-mortem normal-appearing white matter (NAWM) and normal-appearing cortical grey matter (NAGM) MS tissue as well as control white matter (WM) and cortical grey matter (GM).

First, we showed a conserved expression of top enriched mouse microglia signature genes in human microglia. Next to the well-known signature genes *P2RY12* and *TMEM119*, *ADGRG1* (encoding GPR56) was identified as a marker highly expressed on microglia and nearly absent on macrophages. Second, we demonstrated profound microglial differences between GM and WM regions in both the control (453 differentially expressed (DE) genes) and MS groups (124 DE genes), related to immune regulatory pathways. Microglia isolated from NAGM MS tissue highly expressed genes involved in glycolysis and iron homeostasis, indicative for iron accumulation in microglia, which is an early pathological hallmark for MS. In contrast, microglia from NAWM MS tissue showed increased expression of genes implicated in lipid metabolism and lysosomal functioning, suggesting early signs of demyelination. We confirmed that increased expression of lipid metabolism and lysosomal genes are early signs of MS pathology, since IRF8-nuclei in MS lesions showed similar upregulation of these genes. Except for *ADGRG1*, the microglial homeostatic signature was highly conserved in normal-appearing MS tissue, demonstrating that early pathological alterations in normal-appearing microglia do not affect their homeostatic state. To conclude, we demonstrated a region-specific transcriptional profile for microglia across GM and WM brain regions in both control and MS. Moreover, microglial changes in normal-appearing MS tissue are related to early MS pathology, but does not change their homeostatic state.

The phenotype of microglial clusters, so-called nodules, in both stroke and MS post-mortem NAWM tissue was examined in **Chapter 6**, to assess their putative contribution to MS lesion formation. We observed a higher number of HLA-DR$^+$ nodules in MS NAWM tissue as compared to stroke nodules, and the majority of MS nodules contained microglia that are proliferating. Furthermore, proteins implicated in lipid metabolism and phagocytosis, such as MSR1, CHIT1 and CD11c, were expressed by microglial nodules in both stroke and MS, suggesting uptake and degradation of myelin debris, indicative for demyelination. Microglia within nodules in both stroke and MS also expressed the co-stimulatory molecule CD86, indicating that nodules can regulate T-cell activation. Indeed, only in MS, some nodules were in close contact to parenchymal lymphocytes, and a high percentage of T cells were proliferating. Besides plasma and B cells, we found expression of antibody genes only in MS nodule tissue. Finally, MS brain donors with nodules had a higher number of reactive sites and total WM lesions, as well as a higher proportion of active MS lesions, as compared to stroke nodules. Altogether, phagocytic active nodules, indicative for demyelination, reside in an inflammatory environment, suggesting a role for these nodules in MS lesion formation.

In **Chapter 7**, we showed that tolerance of primary human microglia can be broken by co-stimulation with immunoglobulin (Ig)G-immune complexes and Toll-like receptor (TLR)-ligands. Intrathecal antibodies, detected as oligoclonal bands in the CSF of MS patients, are a hallmark for MS diagnosis. First, we demonstrated that myelin obtained from 8 out of 11 MS brain donors was bound by IgG antibodies. Importantly, these antibodies could form immune complexes and break microglial tolerance for microbial stimuli, such as LPS and Poly I:C, causing increased production of pro-inflammatory cytokines, such as TNF, IL-1β and IL-8. In addition, IgG receptors FcγRI and FcγRIIa were responsible for breaking of microglial immune tolerance. Concluding, these data showed that antibody complexes bound to MS myelin potentiate microglial inflammation, by increased expression of pro-inflammatory cytokines and chemokines, which might contribute to the formation of

MS lesions.

The molecular mechanisms implicated in MS lesion activity were assessed by whole tissue gene-expression profiling of the rim and peri-rim of chronic (mixed) active and inactive MS lesions and compared to control WM tissue (**Chapter 8**). Cluster analysis revealed three genes that were specifically upregulated around chronic active lesions: *NCAN*, *TKTL* and *ANO4*. Furthermore, genes related to phagocytosis and lipid processing, including *MSR1*, *CD68*, *CXCL16*, *OLR1*, *CHIT1*, *GPNMB* and *CCL18*, were highly expressed in the rim of chronic active lesions, which contain many foamy microglia/macrophages. Interestingly, some of these genes were already highly expressed around chronic active lesions, indicating that these lesions are expanding. Furthermore, we demonstrated that *CHIT1* and *GPNMB* are implicated in uptake and processing of MS myelin by macrophages and showed that scavenger receptors MSR1 and CXCL16 reduce uptake of myelin *in vitro*. Combined, genes associated with lipid uptake and processing are already present around chronic active MS lesions, indicative for early demyelination and demonstrate that these lesions are expanding.

The contribution of microglia and macrophages to active lesion formation in progressive MS is still unclear. To assess their phenotype in active lesions, we isolated myeloid cells from ten progressive MS cases and compared their phenotype between active lesions and normal-appearing white matter tissue (from the same donors), determined by single cell multiplex mass cytometry (CyTOF) in **Chapter 9**. We used 74 markers to phenotype myeloid cell clusters in active MS lesions, which showed a decreased expression of homeostatic proteins, including TMEM119, P2RY12 and GPR56. Furthermore, lesion-enriched myeloid cells demonstrated a phagocytic and activation-associated phenotype, but lack expression of pro-inflammatory cytokines. The phenotype of normal-appearing myeloid cells is comparable to control myeloid cells. Interestingly, hardly any infiltrating monocyte-derived macrophages were found in active lesions of progressive MS donors. Concluding, in progressive MS, active lesions contained myeloid cell clusters with an activated, phagocytic phenotype, but they are noninflammatory and infiltrating monocyte-derived macrophages were hardly present.