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Macroglial diversity and its effect on myelination

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Macroglial diversity and its effect on myelination

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Introduction and scope of thesis

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Introduction and scope of thesis

Introduction

The central nervous system (CNS) contains neurons, microglia and macroglia, the latter comprising astrocytes (ASTRs) and oligodendroglial cells. Oligodendrocytes (OLGs) mature from oligodendrocyte progenitor cells (OPCs) and ensheath axons with myelin, which is a stack of several lipid bilayers that facilitates saltatory conduction and provides metabolic axonal support^{1,2}. In the demyelinating disease multiple sclerosis (MS), OLGs and myelin are lost, which is accompanied by inflammation, astrogliosis and neurodegeneration, and leads to progressive neurological disability³⁻⁵. Remyelination is a natural process following demyelination and requires the generation of new myelin sheaths, which is essential for functional recovery and preventing irreversible neurological symptoms⁴. Unfortunately, remyelination in MS is often limited and ultimately fails as the disease progresses^{4,6-8}. In experimental models it is shown that remyelination is a multistep process that involves the sequential activation of adjacent OPCs, recruitment of OPCs towards the demyelinated area, and OPC maturation within the demyelinated area^{4,9-11}. While in robust rodent models remyelination is performed by newly-formed OLGs⁴, in MS OPCs are relatively quiescent^{12,13}, and remyelination is performed by pre-existing, mature OLGs¹⁴. Whether remyelination by pre-existing OLGs is an adaptation of the inability of OPCs to mature to OLGs, or a natural process remains to be determined. The process of remyelination is orchestrated among others by transient signaling from ASTRs. Upon injury, such as upon OLG loss and demyelination, ASTRs become reactive, which involves ASTR proliferation, upregulation of specific proteins, including filament proteins GFAP and vimentin, and the elaboration of a dense network of processes¹⁵⁻²⁰. Two subtypes of reactive ASTRs have been described, anti-inflammatory A₂-ASTRs and pro-inflammatory A₁-ASTRs. Mild activation of ASTRs may induce a pro-reparative A₂ phenotype, while reactive A₁-ASTRs, which are observed in MS²¹, inhibit OPC proliferation, migration and differentiation, and are in addition toxic to mature OLGs²¹⁻²⁴. Moreover, in MS, reactive ASTRs form an astroglial scar around inflammatory WM lesions, among others by the generation of a dense network of extracellular matrix proteins, which is considered detrimental for remyelination²⁵. ASTR reactivity is regulated by pro-inflammatory cytokines and Toll-like receptor (TLR)-mediated signaling events, as well as myelin debris^{23,26-29}. Of

importance, pro-inflammatory cytokines, including IL1 β , IFN γ and TNF α ³⁰⁻³², and endogenous TLR agonists³³⁻³⁶ are abundantly present in MS lesions, and TLR₃ and TLR₄ are upregulated on reactive ASTRs within MS lesions²⁹.

Remarkably, in MS as well as in experimental models remyelination is more robust in grey matter (GM) areas than in white matter (WM) areas^{17,18,37,38}. Of special interest in this regard are leukocortical lesions in MS, which span both the GM and WM. These lesions are thought to have the same pathological age and background. Within these lesions, more remyelination is observed in the GM area of the lesion compared to the WM area of the lesion³⁷. Differences in regional remyelination can be caused by both intrinsic differences in OPCs, OLGs and/or differences in extrinsic signals derived from, among others, ASTRs. For example, in experimental demyelination models, ASTR reactivity is more prominent in the corpus callosum, a WM area, than in the cortex, a GM area^{15-17,39}. Indeed, macroglia form distinct populations across different brain regions^{12,40,41}. Whereas particularly OLGs appear to form a heterogeneous group of cells based on their transcriptional profile⁴², ASTR are morphologically diverse, especially in GM and WM areas, and have a high functional plasticity when adapting to the specific needs of the local micro-environment^{43,44}. This may result in subsequent ASTR regional diversity due to adaptation to the demands of cells in the region. Of importance, heterogeneity and plasticity of macroglia will affect the response to injury and affect recovery, thus contributing to the pathology. Notably, most therapies for MS do not directly aim at promoting remyelination, but rely on disease-modifying treatments, involving an alteration of the immune response and a diminishment of the number and severity of attacks⁴⁵. Hence, elucidation of macroglial diversity in GM *versus* WM, and its alleged contribution to the observed differences between GM and WM with regard to remyelination efficiency may open novel therapeutic avenues aimed at enhancing remyelination in MS.

Scope of thesis

The aim of the work described in this thesis was to explore potential differences in macroglia in GM and WM, and if so, whether and how this affects (re)myelination. To address the issue whether regional macroglia differ in their ability to modulate processes that are relevant for (re)myelination, primary ASTRs and OLGs are used, as well as an *in vitro* myelinating culture system that depends on a feeding layer of

ASTRs. In **chapter 1**, current knowledge of macroglia diversity in CNS GM and WM is reviewed and discussed in the context of whether and how pre-existing heterogeneity and plasticity contribute to successful and failed remyelination, the latter being a major cause of disease progression in MS. This literature overview highlights several issues that are discussed in the context of the work presented in this thesis, including the importance of macroglia interactions in remyelination. In **chapters 2, 3, and 4**, heterogeneity and plasticity between gmASTRs and wmASTRs and differences in their potential to modulate OPC behavior and *in vitro* myelination, are investigated. A previously identified extracellular matrix protein, fibronectin, forms aggregates which persist in MS lesions and inhibit remyelination⁴⁶. Therefore, **chapter 2** focusses on the underlying mechanism of the formation of these remyelination-inhibiting fibronectin aggregates by ASTRs. Using primary neonatal rat ASTRs the role of pro-inflammatory cytokines, TLR agonists and fibronectin splice variants on fibronectin aggregate formation was examined, taking into account potential differences between gmASTRs and wmASTRs. In **chapter 3**, we first determined whether primary neonatal gmASTRs and wmASTRs differ in their capacity to modulate *in vitro* myelination. Cholesterol is an essential, major integral lipid of myelin membranes⁴⁷. Presumably, during development and likely also upon demyelinating injury, cholesterol is supplied to myelinating OLGs by ASTRs and subsequently incorporated into the myelin membrane⁴⁸. Therefore, potential differences in cholesterol production and influx into wmASTR versus gmASTRs were examined and whether such differences could distinctly modulate myelination. In addition, the effects of pro-inflammatory cytokines and TLR agonists on astrocyte-mediated cholesterol efflux were investigated, as well as the identification of the cholesterol transporters that contribute to the lipid's efflux. In **chapter 4**, a 3'-RNA-sequencing study was carried out to clarify whether cultured adult gmASTRs and wmASTRs were heterogeneous cell populations that distinctly modulate *in vitro* myelination. To reveal transcriptionally different regulatory mechanisms between gmASTRs and wmASTRs that may translate to differences in their modulation of myelination, a weighted gene network co-expression analysis of the obtained sequencing data was performed. In addition, the effects of secreted soluble factors and potential deposits of extracellular matrix proteins on primary OPCs were investigated, and if so, whether and how these effects were affected upon TLR agonist treatment of ASTRs. In addition to ASTRs, also OPCs in the GM and WM may differ in their ability to myelinate, and

thus contribute to differences in remyelination in GM and WM, which is explored in **chapter 5**. Here, differences between gmOPCs and wmOPCs were studied in terms of proliferation, migration, differentiation and myelin membrane formation, as well as their sensitivity to pro-inflammatory cytokines. **Chapter 6** summarizes and discusses the work presented in this thesis in light of its relevance to MS pathology and the development of remyelination-based therapies in MS.