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Multiple Sclerosis: Glial Cell Diversity in Time and Space

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ABSTRACT

Multiple sclerosis (MS) is the most prevalent human inflammatory disease of the central nervous system with demyelination and glial scar formation as pathological hallmarks. Glial cells are key drivers of lesion progression in MS with roles in both tissue damage and repair depending on the surrounding microenvironment and the functional state of the individual glial subtype. In this review, we describe recent developments in the context of glial cell diversity in MS summarizing key findings with respect to pathological and maladaptive functions related to disease-associated glial subtypes. A particular focus is on the spatial and temporal dynamics of glial cells including subtypes of microglia, oligodendrocytes, and astrocytes. We contextualize recent high-dimensional findings suggesting that glial cells dynamically change with respect to epigenomic, transcriptomic, and metabolic features across the inflamed rim and during the progression of MS lesions. In summary, detailed knowledge of spatially restricted glial subtype functions is critical for a better understanding of MS pathology and its pathogenesis as well as the development of novel MS therapies targeting specific glial cell types.

1 | Introduction

As the name suggests, multiple sclerosis (MS) is a multifaceted disease that exhibits an enormous level of heterogeneity at different scales. MS is a chronic inflammatory, demyelinating and eventually degenerative disease affecting both white and gray matter areas (Kutzelnigg et al. 2005; Haider et al. 2016). In most cases, MS starts as a relapsing–remitting disease that gradually transitions into a progressive phase, where symptoms gradually worsen without distinct relapses and remissions. Most pathological studies focus on brains from individuals with long-standing, progressive disease (Mahad, Trapp, and Lassmann 2015); however, such studies may overlook early pathophysiological events as seen in MS biopsy studies (Lucchinetti et al. 2011). The disease is typically characterized by the accumulation of focal lesions with variable levels of inflammation that can basically appear everywhere across the central nervous system (CNS). MS is a disease with a profound level of inter-individual heterogeneity in symptoms, clinical progression and, importantly, differential response patterns to treatment interventions including pharmacological therapies.

Heterogeneity also exists with respect to tissue damage and resilience factors between individuals. For example, whereas some patients show a high degree of brain atrophy early after disease onset, others have a mild course with only little evidence for brain atrophy and neurodegeneration, notably, even many years after

initial symptoms. Individual differences in clinical worsening and between outcomes are challenging to measure. However, certain genetic variants as shown for single nucleotide polymorphisms are associated with increased susceptibility (International Multiple Sclerosis Genetics Consortium 2019) and clinical severity as risk factors in individuals affected by MS (International Multiple Sclerosis Genetics Consortium and MultipleMS Consortium 2023). Other factors with varying impacts on the individual disease course are environmental ones such as the microbiome (Jangi et al. 2016; Pröbstel et al. 2020; Cox et al. 2021) or the exposure to light as shown for vitamin D (Ascherio, Munger, and Simon 2010) as a potential resilience factor in MS. Other external factors are related to viral infections during adolescence as shown for Epstein–Barr virus (Bjornevik et al. 2022; Cortese et al. 2024) that might trigger autoimmune responses against glial proteins through processes such as molecular mimicry (Lanz et al. 2022).

1.1 | Heterogeneity of MS Lesion Pathology

The concept of heterogeneity in clinical presentation is also resembled in the pathology of the disease, where acute inflammatory lesions are regularly found side-by-side to chronic less inflammatory lesions with a varying pattern of microglial activation. Of note, acute lesions are associated with a high density of myelin-phagocytosing myeloid cells not only at border areas but throughout the entire lesion. Although the precise mechanisms underlying acute tissue damage and loss of oligodendrocytes are still unclear, it has been proposed that such early active lesions can be divided in different types based on histopathological criteria (Lucchinetti et al. 2000) that persist over time (Metz et al. 2014). Of note, subtyping of acute lesions only applies to lesions in subcortical white matter of a very early inflammatory stage. Hence, other studies aimed at characterizing lesions at more advanced stages to better understand lesion progression and, for example, measure the impact of neuroaxonal damage over time (Mahad et al. 2009; Schirmer et al. 2011; Oost et al. 2023). Moreover, other work focused on spatially defined tissue compartments such as the lesion rim (Absinta et al. 2021) or defined cortical layers (Kaufmann et al. 2022). For example, it is known that demyelination of subpial upper cortical layers is a common feature of MS pathology and much more prevalent than demyelination of deeper cortical layers (Albert et al. 2007; Lucchinetti et al. 2011). However, it is still unknown if the sole driver of subpial pathology is sustained meningeal inflammation or other factors such as cumulative damage to subcortical fiber tracts or selective levels of glial subtype neurotoxicity more prevalent in upper cortical layers.

The mosaic of different lesion types in MS is reflected by a high level of cellular heterogeneity, which is driven by a mix of various homeostatic cell types and lesion-associated reactive subtypes. With the advent of single-cell techniques to decode transcriptomic signatures of individual cells in postmortem brain tissues (Lake et al. 2016; Schirmer et al. 2019; Velmeshev et al. 2019), it has become evident that glial cell type diversity is even more complex. Under physiological conditions, glial cell types show a high level of heterogeneity with respect to morphological, functional and molecular properties between anatomical areas such as between white and gray matter or between different CNS areas such as the cerebellum or the spinal cord (Trobisch et al. 2022; Siletti et al. 2023). Thus, it is a key to understand the physiological level of cell type heterogeneity prior to studying the even more diverse aspects of glial subtype reactivity under pathological conditions such as in MS.

1.2 | New Technologies to Subtype Glial Cells in MS Research

Single-cell transcriptomics applied to MS brains has helped understand anatomical differences between glial cells comparing gray (Schirmer et al. 2019) with white matter areas (Jäkel et al. 2019; Absinta et al. 2021) or distinct regions such as the cerebellum or the spinal cord (Trobisch et al. 2022; Seeker et al. 2023). It is likely that the regional identity of glial subtypes determines the reactivity level during inflammation and demyelination. Typical rim lesions, for example, commonly found in subcortical white matter and characterized by a wall of swollen and highly reactive astrocytes are usually not found in cortical gray matter areas. Also, it has been speculated that cortical lesions might be more efficient to remyelinate (Albert et al. 2007). Hence, it would be plausible to suggest that spatially restricted glial subtypes are key contributors determining the fate and outcome of tissue damage and lesion pathology. Regional diversity has been shown for the oligodendrocyte lineage (Marques et al. 2018; Spitzer et al. 2019), and it has been speculated that differences in regional identity would explain selective patterns of oligodendrocyte vulnerability in MS (Trobisch et al. 2022; Seeker et al. 2023). For example, it is a typical feature of myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) that demyelination preferentially occurs along the optic nerves and the spinal cord, similar to neuromyelitis optica (NMO), which is associated with serum antibodies against aquaporin-4 (AQP4). As both regions are rich in white matter tracts, one idea could be that oligodendrocyte and astrocyte subtypes associated with white matter might be more vulnerable and prime targets of humoral immune responses in both diseases. Furthermore, diversity of myeloid cells including CNS-associated macrophages versus tissue resident microglia as shown in human and mouse tissues (Sankowski et al. 2024) might have direct influence on the degree of tissue damage and eventually disease outcome. One emerging example in MS are border-associated myeloid cells that represent a diverse population of cells with distinct functions in promoting versus limiting further tissue damage and lesion expansion.

More recently, spatially resolved omics including spatial transcriptomics and spatial epigenomics as well as proteomics have helped precisely map cell type-specific signatures to coordinates across physiological and disease tissue areas. The latter tools have been critical as they now allow studying the molecular properties of individual cell types as well as their appearance in space. Those technological advances were accompanied by the development and use of powerful computational tools that enabled us to investigate and decode cell–cell communication events and molecular changes between various cell types as well as spatially restricted tissue niches.

In this review, we highlight recent studies that have used single-cell or single-nucleus RNA sequencing or spatial transcriptomics to explore spatial and cellular diversity related to glial cell types associated with MS lesion composition and progression (Table 1). In addition, we will discuss future directions for cell

TABLE 1 | Single cell and spatial transcriptomics of human MS tissue. Overview of transcriptomic studies using single-cell/nucleus and spatially resolved work on human MS tissue. All available datasets between 2019 (first study published) and November 2024 were included.

	Name first author	Year of publication	Techniques used	Species	Sample size	Age range (years)
Single cell/single nucleus RNA sequencing of MS lesions	Jäkel	2019	snRNA-seq (10×)	Human	Controls: 5, MS: 4	Controls: 35–82, MS: 37–57
	Schirmer	2019	snRNA-seq (10×)	Human	Controls: 9, MS: 12	Controls: 34–82, MS: 34–55
	Masuda	2019	scRNA-seq (Cel-seq2) on sorted CD45 + cells	Human	Controls: 5, MS: 5	Controls: 22–54, MS: 27–40
	Wheeler	2020	scRNA-seq (Drop-seq) on sorted myeloid cells	Human	Controls: 5, MS: 4	Controls: 45–62, MS: 26–65
	Absinta	2021	LCM, snRNA-seq (10×)	Human	Controls: 3, MS: 5	Controls: 49–60, MS: 44–56
	Miedema	2022	scRNA-seq (10×) on sorted myeloid cells	Human	Controls: 0, MS: 5	MS: 56–67
	Trobisch	2022	snRNA-seq (10×)	Human	Controls: 3, MS: 3 (new samples integrated with leukocortical samples from Schirmer et al. 2019)	Cerebellum—controls: 35–64, MS: 55–64; spinal cord—controls: 61–90, MS: 71–81
	MacNair	2024	snRNA-seq (10×)	Human	Controls: 26, MS: 54	N/a in detail, graphical representation is available in indicated doi
	Seeker	2023	snRNA-seq (10×)	Human	Controls: 20, MS: 0 (integrated with leukocortical samples from Jäkel et al. 2019)	Young: 34–45, old: 61–74
	Tuddenham	2024	scRNA-seq on sorted CD45 + cells	Human	MS: 1, other diseases and biopsy controls: 73	MS: 47
Spatial transcriptomics of MS lesions	Kaufmann	2022	ST (custom made, resolution 200 μm)	Human	Controls: 5, MS: 32	Controls: 35–82, MS: 35–65
	Lam	2023	Visium (10×, resolution 55 μm), snRNA-seq (10×)	Human	Controls: 15, MS: 8	NA
	Sankowski	2024	ISS (Cartana, cellular resolution), CosMx (Nanostring)	Human	ISS: controls: 1, MS: 0 CosMx: controls: 1, MS: 0	ISS: age range 21–40; CosMx: age range 61–80
	Kukanja	2024	ISS (Cartana, cellular resolution)/ Xenium (10×, cellular resolution)	Mouse/ human	Human: controls: 2, MS: 4	Controls: 57–72, MS: 47–66
	Alsema	2024	Visium (10×, resolution 55 μm)/ISS (Cartana)/GeoMx (Nanostring)	Human	Controls: 5, MS: 21/ controls: 1, MS: 2 /controls: 0, MS: 5	Controls: 60–85, MS: 35–76/ control: 60, MS: 35, 66/ control:-, MS: 51–81
	Lerma-Martin	2024	Visium (10×, resolution 55 μm), snRNAseq (10×)	Human	Controls: 7, MS: 21	Controls: 54–84, MS: 42–61

TABLE 1 | (Continued)

	Sex M:F	CNS region	Lesion type	Most significant findings	Reference (DOI)
Single cell/single nucleus RNA sequencing of MS lesions	Controls 4:1, MS: 3:1	WM	Leukocortical (NAGM, GML, NAWM, WML incldluing remyelination)	Differences in mature oligodendrocyte subclusters in MS lesions. Similar changes were observed in NAWM, suggesting that MS is a more diffuse disease than its focal demyelinated areas	10.1038/s41586-019-0903-2
	Controls: 5:4, MS: 4:8	WM, GM	Leukocortical (NAGM, GML, NAWM, WML)	Lineage- and region-specific transcriptomic changes associated with selective cortical neuron damage and glial activation contributing to MS lesion progression	10.1038/s41586-019-1404-z
	Controls: 3:2, MS: 2:3	WM (MS), GM (controls)	Leukocortical biopsies (early active lesions)	Flow cytometric isolation of CD45+ microglia and macrophages from early active MS lesions revealed MS-specific subtypes that could be mapped to MS lesion areas	10.1038/s41586-019-0924-x
	Controls: 4:1, MS: 3:1	WM, GM	Freshly processed leukocortical tissues (exact lesion location and activity not defined)	Subtype-specific characterization of MS astrocytes with pro-inflammmatory properties; astrocytes have decreased expression of NRF2 and increased expression of MAFG, linked to regulatory functions	10.1038/s41586-020-1999-0
	Controls: 2:1, MS: 4:1	WM	Subcortical (NAWM, WML: active/inactive lesions)	Spatially defined astrocytes and microglia inflamed in MS at the lesion rim	10.1038/s41586-021-03892-7
	MS: 2:3	WM, GM	Leukocortical (NAGM, GML, NAWM, WML)	Flow cytometric CD45 + CD11B+ isolation of microglia and macrophages revealed MS lesion-associated cells with increased cellular stress in microglia in NAWM	10.1186/s40478-021-01306-3
	Cerebellum—controls: 2:1, MS: 1:2; spinal cord—controls: 1:2, MS: 0:3	WM, GM	Leukocortical, cerebellum, spinal cord (NAGM, GML, NAWM, WML)	Patterns of transcriptomic changes in MS are shared across CNS regions and converge on specific pathways, especially those regulating cellular stress and immune activation	10.1007/s00401-022-02497-2
	WM—control: 9:7, MS: 14:20; GM—control: 10:7, MS: 11:10	WM, GM	Leukocortical (NAGM, GML, NAWM, WML)	Identification of 4 MS subgroups with distinct WM glial gene expression signatures and patterns of oligodendrocyte stress and/or maturation; patterns provide a framework to use molecular biomarkers to stratify patients	10.1016/j.neuron.2024.11.016

(Continues)

TABLE 1 | (Continued)

	Sex M:F	CNS region	Lesion type	Most significant findings	Reference (DOI)
	Young: 5:5, old: 5:5	WM, GM	Leukocortical, cerebellum, spinal cord (NAGM, GML, NAWM, WML)	Distinct transcriptomic signatures between CNS regions with microglia cells showing a stronger inflammatory signature in spinal cord tissue	10.1186/s40478-023-01568-z
	ms: 1:0	Thalamus, GM, aanterior watershed	NA	Identification of common microglia signatures accross diseases	10.1038/s41593-024-01764-7
Spatial transcriptomics of MS lesions	Controls: 5:0, MS: 2:11	GM	Cortical (NAGM, GML)	Multicellular mechanisms of progressive MS pathogenesis with an origin related to spatially distributed stages of neurodegeneration	10.1038/s41593-022-01097-3
	Controls: 6:9, MS: 4–4	DLPFC, PULV	Leukocortical (NAGM, NAWM)	Cell type- and disease-specific differences present even in non-lesional white and gray matter tissue	10.1101/2023.12.20.572491
	ISS: 1:0; CosMx: 1:0	CNS interfaces	Different inferfaces including leptomeninges	Comprehensive overview of myeloid diversity at the interfaces of the human CNS with the periphery	10.1038/s41591-023-02673-1
	Controls: 1:1, MS: 2:2	Brain, spinal cord/ WM	MS (spinal cord NAGM, GML, NAWM, WML: active/inactive lesions)	A model for centrifugal development of lesions in EAE with a key role for disease associated glia	10.1016/j.cell.2024.02.030
	Controls: 2:3, MS: 8:13/ control: F, MS: 0:2 / control:-, MS: 2:3	WM, GM	Leukocortical (NAGM, GML, NAWM, WML: active, active/inactive)	A novel transcriptionally distinct rim in active lesions and a predicted model for lesion evolution from NAWM to active/ inactibe lesions where the rim is identified as the key region for lesion evolution	10.1038/s41593-024-01765-6
	Controls: 3:4, MS: 3:5	WM	Subcortical (NAWM, WML: active/inactive lesions)	Insights into the conversion of the tissue microenvironment from a “homeostatic’ to a pathogenic or ‘dysfunctional” state underlying lesion progression in MS.	10.1038/s41593-024-01796-z

Abbreviations: CNS: central nervous system, DLPFC: dorsolateral prefrontal cortex, EAE: experimental autoimmune encephalomyelitis, GM: gray matter, GML: gray matter lesion, ISS: in situ sequencing, LCM: laser capture microdissection, NA: not available, NAGM: normal appearing gray matter, NAWM: normal appearing white matter, PULV: pulvinar (thalamus), scRNA-seq: single-cell RNA sequencing, snRNA-seq: single-nucleus RNA sequencing, ST: spatial transcriptomics, WM: white matter, WML: white matter lesion.

type and spatial omics research and omics-based data integration with an emphasis on MS and related neurological diseases.

2 | Glial Cell Type Diversity and Reactivity

2.1 | Myeloid Cell Subtype Diversity

The CNS is home to several myeloid cell types that under homeostatic conditions occupy distinct spatial domains within the CNS. While microglia reside in the parenchyma, several types of border-associated macrophages (BAMs) are recognized including perivascular macrophages, macrophages surrounding the choroid plexus and macrophages occupying the subdural space (Mildenberger, Stifter, and Greter 2022; Dalmau Gasull et al. 2024).

Microglia, as resident cells in the parenchyma, plays vital roles in shaping the CNS during development. They colonize the CNS prior to neurogenesis, myelination and formation of the BBB (Monier et al. 2006, 2007; Menassa and Gomez-Nicola 2018) and, among others, contribute to the formation of synaptic networks (Paolicelli et al. 2011; Zhan et al. 2014) in addition to maintaining structural integrity during morphogenesis (Lawrence et al. 2024). In adulthood, microglia express a plethora of receptors that are used to sense changes in their surrounding microenvironment (Hickman et al. 2013; Galatro et al. 2017; Gosselin et al. 2017) allowing them to rapidly respond to any pathological process in an effort to limit damage and maintain CNS homeostasis. Already when they were first described, it was recognized that microglia can exist in different morphological states, depending on the environment (Sierra et al. 2016). Given their diversity in function, it is maybe not surprising that microglia are a highly heterogeneous population whose diversity depends on many aspects including factors like developmental stage (Matcovitch-Natan et al. 2016; Bian et al. 2020; Kracht et al. 2020), age (Li et al. 2019; Marschallinger et al. 2020), brain region (Grabert et al. 2016; Böttcher et al. 2019), sex (Thion et al. 2018), and disease status (Krasemann et al. 2017; Srinivasan et al. 2020; Absinta et al. 2021; Gerrits et al. 2021; Miedema et al. 2022; Paolicelli et al. 2022).

Microglia and BAMs can be distinguished based on their location in homeostasis, but also recent single-cell and single-nucleus transcriptomics work, has provided a framework with markers for each specific type of CNS macrophage (Mildenberger, Stifter, and Greter 2022; Muzio and Perego 2024). Microglia typically show a high expression level of markers such as TMEM119, SALL1, CX3CR1, and P2RY12, while BAMs have their own unique signatures including, but not limited to, expression of LYVE1, CD163, and MRC1 (Goldmann et al. 2016; Mrdjen et al. 2018; Van Hove et al. 2019; Utz et al. 2020). In line with their capability to sense a large diversity of signaling molecules, locally derived environmental cues drive the occurrence of distinct myeloid cell types in the CNS (Gosselin et al. 2014, 2017; Lavin et al. 2014; Masuda et al. 2022). In the context of MS, microglia can adopt several disease-associated subtypes linked to different functional properties (Figure 1). In particular,

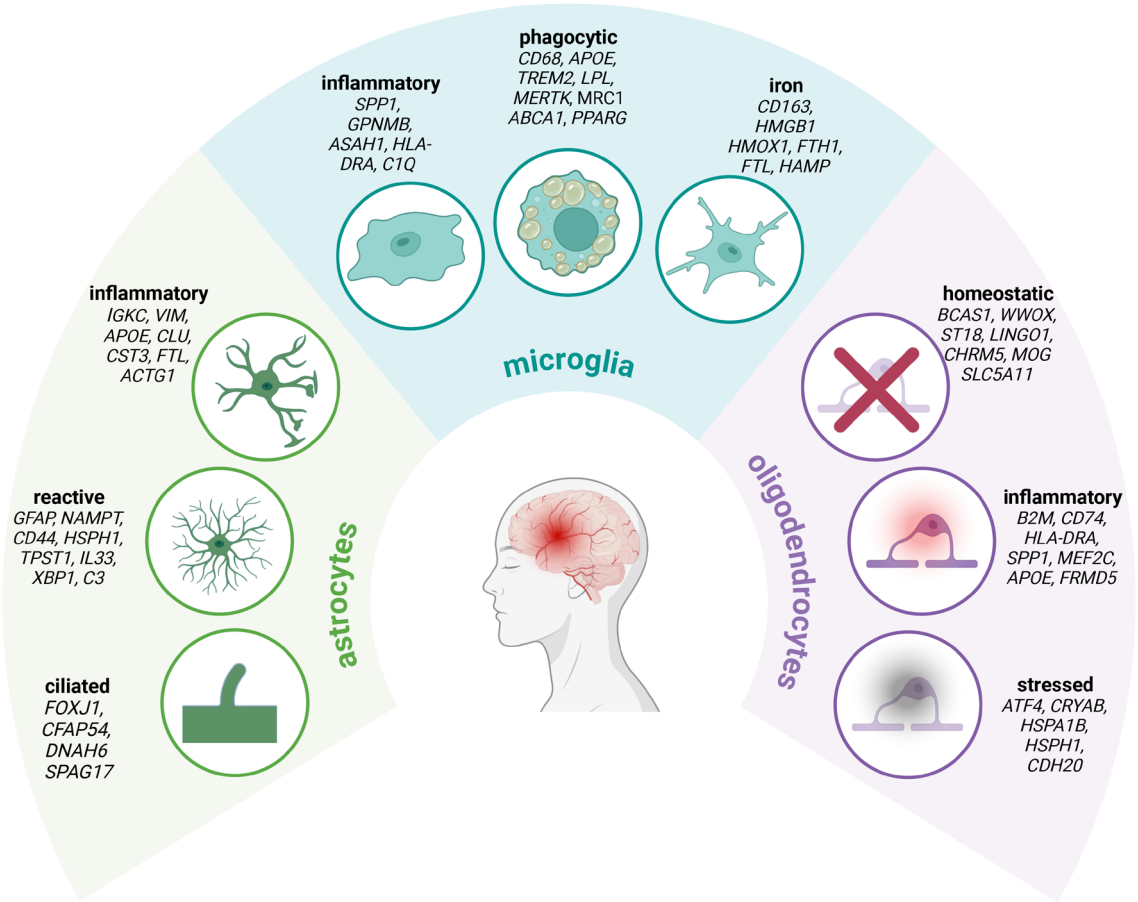

FIGURE 1 | Spectrum of glial cell diversity in MS. A selection of disease-associated subtypes of glial cells identified in human MS tissue in the context of white matter lesions is indicated including several marker genes for these populations. Created with BioRender.com

transcriptomic changes have been observed in microglia that is associated with lesions (Masuda et al. 2019; Absinta et al. 2021; Miedema et al. 2022). Microglia in MS lesions are characterized by gene expression profiles related to biological processes like phagocytosis and lipid metabolism, which appears in agreement with the role of microglia in clearance of myelin debris (Forbes and Miron 2022). The observed signature consists of changes in the expression of GPNMB and SPP1 and shows substantial overlap with microglia signatures observed in classic neurodegenerative diseases.

These signatures are often attributed to disease associated microglia (DAM), whose occurrence was described to follow a step-wise transition that is partially dependent on TREM2 (Keren-Shaul et al. 2017). Initially identified in a mouse amyloid model, the DAM signature has been challenging to capture in human microglia, though at least a partial overlap has been observed both in Alzheimer's disease and MS, which includes the expression of critical marker genes like *TREM2* and *APOE* (Gerrits et al. 2021; Miedema et al. 2022). Typically, increased expression of DAM genes is associated with decreased expression of homeostatic genes. To robustly identify common human microglia signatures, a recent study described the single-cell RNA sequencing analysis of a large number of CNS myeloid cells across many diseases, including cells isolated from a limited number of postmortem MS brain tissues (Tuddenham et al. 2024). Other diseases included were Alzheimer's disease, frontotemporal dementia, amyotrophic lateral sclerosis, and Parkinson's disease among others. Exploring the approximately 250,000 collected microglia profiles, there seems to be a shared transcriptional response of microglia in the context of these neurological diseases, as 12 microglial subpopulations were identified that were represented across all diseases and regions (Tuddenham et al. 2024). Interestingly, the most common microglial subtypes in most individuals shared a homeostatic gene expression signature. Cells isolated from donors with neurological diseases were characterized by shifts toward distinct microglial subtypes. Several of these subtypes, whose marker genes suggest a shift in function towards heterocyclic metabolism and nitrogen-containing compound metabolism as well as transcriptional regulation and motility, were significantly associated with the genetic risk for MS, suggesting these subtypes are linked to MS susceptibility (Tuddenham et al. 2024).

However, as indicated above, there is heterogeneity in lesion types, as well as in cellular composition across lesion areas, including spatially defined variation in abundance and appearance of microglia (Kuhlmann et al. 2017; Luchetti et al. 2018). Using MRI-guided snRNA-seq at the rim of mixed active/inactive lesions, Absinta et al. identified a myeloid cell population linked to a strong inflammatory gene set signature that they annotated as "microglia inflamed in MS" (MIMS). Of note, in that study two types of MIMS were described: one of the identified MS microglia subtypes, MIMS-foamy, had an increase in genes associated with pathways such as lipid storage and response to lipoprotein particles, suggesting that these cells contribute to phagocytosis and clearance of myelin debris (Absinta et al. 2021); the other MS-related subtype of myeloid cells had a strong iron uptake signal and was annotated MIMS-iron. This subtype was associated with a gene set signature linked to iron metabolism with markers such as ferritin, but also shared genes related to antigen presentation, as well as other inflammatory markers like IL1B and C1q. In a follow-up study, Hofmann et al. could further characterize this subtype and found that the haptoglobin–hemoglobin scavenger receptor CD161 and downstream markers involved in intracellular iron metabolism and export functions such as HO-1 (encoded by *HMOX1*) or hepcidin (encoded by *HAMP*) were upregulated (Hofmann et al. 2023). Overall, there is increasing evidence that iron uptake and inflammatory response patterns might be closely linked to each other in a subtype of MS-specific myeloid cells, however, whether those cells derive from blood borne monocytes or resident microglia needs to be elucidated.

Overall, it has been demonstrated that the state of microglia observed in MS lesions is at least partially dependent on the lesion type and localization to specific lesion areas. However, besides changes associated with demyelinated areas in the MS brain, the transcriptome of microglia in the normal appearing white matter in MS donors is already affected, with microglia displaying increased expression of genes associated with cellular stress (Miedema et al. 2022). Interestingly, it has been long known that small clusters of activated microglia, microglia nodules, can be observed in the MS brain without apparent demyelination (Van Horssen et al. 2012). Recently, it was described that microglia in these nodules express genes associated with lipid metabolism, presence of T and B cells, production of immunoglobulins and cytokines, activation of the complement cascade, and metabolic stress (Van den Bosch et al. 2024). Interestingly, in some cases, the nodules were associated with the presence of partially demyelinated axons, suggesting that the microglia in these nodules contribute to the initiation of MS lesion formation (Van den Bosch et al. 2024). To what degree the observed signature in microglia in the overall NAWM and specifically in nodules contribute to initiation of lesion formation or represent a gene expression profile geared toward protective mechanisms and limiting damage remains to be determined.

As for BAMs, though recent work has delineated their origins and phenotypes under homeostasis, how they contribute to the pathophysiology of MS remains largely unresolved. In mouse models of MS, it has been demonstrated that brain resident macrophages, including meningeal macrophages, are not required to initiate CNS inflammation (Mundt et al. 2019); (Wolf et al. 2018). In view of their position at the interfaces between the brain and the periphery, they might be involved in immune surveillance, regulating vascular permeability and maintaining CSF, and CNS homeostasis (Mildenberger, Stifter, and Greter 2022).

An added layer of complexity to the myeloid landscape in MS is that during certain stages of lesion development, monocytes, and macrophages can cross the BBB and migrate into the CNS as well. Of note, microglia can change shape and start to express typical "macrophage" transcripts in the context of MS and, conversely, brain-infiltrating myeloid cells start to adopt a "microglial" signature due to local environmental cues, which makes tracing of and distinguishing these populations in the diseased brain extremely challenging. Here the advent of single-cell and single-nucleus RNA-seq has contributed majorly to our understanding of these diverse cell types (Mildenberger, Stifter, and Greter 2022; Amann, Masuda, and Prinz 2023). Studies

have found that the overall transcriptome of infiltrated cells remains significantly different from the resident cells (Shemer et al. 2018), and that infiltrated cells typically are not retained in the CNS long-term (Jordão et al. 2019). In addition, high-plex proteomic analysis of progressive MS samples similarly showed increased abundance of highly phagocytic and activated microglia states in active lesions compared to NAWM and control. Interestingly, in these active lesions from progressive donors, infiltrating monocyte-derived macrophages were scarce; suggesting that the contribution of infiltrating cells is likely dependent on disease duration (Böttcher et al. 2020).

What has become obvious is that for myeloid cells in the context of MS, location matters. It is likely that detailed spatial mapping of myeloid cells and in particular microglia subtypes across different stages of MS lesions will improve our understanding related to their function in lesion development and progression, as well as their roles during scar formation and tissue repair.

2.2 | Astrocyte Subtype Diversity

Astrocytes play critical roles in tissue homeostasis and under pathological conditions as shown for a wide range of neuropsychiatric diseases. For a long time, it has been known that gray and white matter astrocytes differ strongly in terms of function and morphology including changes of cell body as well as the number, form and length of astrocyte processes. With respect to astrocyte reactivity, a major focus has been on white matter GFAP-expressing astrocytes as those cells are more prone to transform into a proinflammatory phenotype with enhanced tissue-damaging functions. In this context, it has been shown that white matter astrocyte reactivity usually stops at the border toward the cortical gray matter (Chang et al. 2012), which generally shows enhanced repair properties that might be linked to a less neurotoxic astrocyte phenotype.

Several years ago, seminal work by Ben Barres and coworkers (Liddelow et al. 2017; Guttenplan et al. 2021) characterized two astrocyte subtypes with either proinflammatory and neurotoxic functions (named A1 astrocytes) versus regulatory and potentially neurotrophic properties (named A2 astrocytes). Also, a population of myeloid cells was identified as the main driver cell type capable of inducing A1 astrocytes through the three key factors interleukin (IL)-1α, tumor necrosis factor α (TNF-α) and complement component 1 subcomponent q (C1q). Based on the expression profile of the A1 subtype, evidence for its existence in humans could be demonstrated for several diseases, including MS. Although this and following studies could carve out key aspects of pro- versus anti-inflammatory astrocytes, such a clean dichotomy with two subtypes having opposing roles in tissue regulation is likely too simplified in the context of a chronic and multifaceted disease like MS. Previous work reported that astrocytes in MS would adopt antigen presenting and phagocytosing functions, potentially supporting myeloid cells in debris clearance. Those studies found astroglial upregulation of MHC class proteins (Ransohoff and Estes 1991), NF-κB activation and uptake of myelin degradation products into astrocytes (Ponath et al. 2017). Along these lines, another elegant study utilizing a novel sequencing approach (SPEAC-seq) to measure cell–cell interaction signatures found amphiregulin secreted by microglial cells to be a negative regulator of astrocyte nuclear factor kappa B activation (Wheeler et al. 2023). Other recent work pairing single-cell with spatial transcriptomics found evidence for a ciliated astrocyte subtype with elongated cilia that appeared in MS lesion core areas (Lerma-Martin et al. 2024) (Figure 1). However, whether those cilia-forming astrocytes resemble an earlier developmental phenotype or might be linked to repair functions needs to be elucidated in future studies.

Other experimental work in MS-related mouse models focused on the crosstalk between astrocytes and immune cell types, which helped identify astrocyte-encoded transcription factors such as XBP1 (Wheeler et al. 2019) and NRF2 (Wheeler et al. 2020) as critical determinants of astrocyte reactivity in experimental neuroinflammation. In another elegant series of experiments, a microglia-astrocyte crosstalk mediated through TGFα and VEGF-B signaling and resulting in a proinflammatory astrocyte switch has been described in the context of experimental inflammatory demyelination (Rothhammer et al. 2018). Although these studies cannot be directly translated to human MS, they provide important insight into signaling pathways triggering proinflammatory astrocyte responses. Further, other studies reported that a loss of resilience factors such as astrocyte-encoded AIM2 function as part of the inflammasome and can exacerbate experimental neuroinflammation (Ma et al. 2021). Of note, as environmental factors such as changes of the gut microbiome can influence clinical worsening in MS, findings from related animal models could highlight the importance of gut-brain signaling in modulating astrocyte subtype function during neuroinflammation. Accordingly, it was shown that microbial metabolites can directly influence astrocyte reactivity through aryl hydrocarbon receptor signaling, and that T cells primed in the gut can modulate astrocyte reactivity suppressing neuroinflammation (Rothhammer et al. 2016). Furthermore, in mice astrocyte-encoded PD-L1, an important immunomodulatory checkpoint, was found to be upregulated in response to aryl hydrocarbon receptor, and the interaction between astroglial PD-L1 and microglia PD-1 was able to control autoimmune CNS inflammation.

In summary, it appears that astrocytes in MS and related models generally lose their physiological and spatially-encoded homeostatic functions and, conversely, adopt proinflammatory and maladaptive properties that are driven by a number of diverse intrinsic (e.g., altered transcription factor activity) and extrinsic (e.g., change in microbiome) factors in the context of chronic inflammation. However, it is unclear how stable astrocyte subtype reactivity is over time considering the progressive nature of MS. Apparently, a recent study in mice found that mouse astrocytes might have an epigenetic memory leading to a pronounced response and activation state upon repeating inflammatory exposure to inflammation (Lee et al. 2024). Hence, in future studies spatial and temporal patterns of astrocyte reactivity need to be studied in more detail to identify and eventually modulate those cells in the context of MS and related disease conditions.

2.3 | Oligodendrocyte Subtype Diversity

Oligodendrocytes, the myelinating cells of the CNS derive from oligodendrocyte precursor cells (OPCs) and represent the major target in MS pathophysiology. scRNA-seq analysis of human

CNS tissues has allowed for the identification of several cellular subtypes in the oligodendrocyte lineage (Marques et al. 2016; Jäkel et al. 2019; Schirmer et al. 2019; Segel et al. 2019; Huang et al. 2020) with likely different functions depending on their site of origin (Trobisch et al. 2022; Seeker et al. 2023). Myelination is a carefully orchestrated process involving the proliferation and differentiation of oligodendrocytes and subsequent formation and wrapping of the myelin sheaths around axons (Bergles and Richardson 2015). While the oligodendrocyte population is relatively stable in adult humans (Yeung et al. 2014), myelin undergoes dynamic modulation throughout life (Osso and Hughes 2024). For example, scRNA-seq has identified heterogeneity in the oligodendrocyte lineage that mainly seems to follow the developmental program associated with different stages of myelination (Van Bruggen, Agirre, and Castelo-Branco 2017). Moreover, sc/snRNA-seq studies of human MS samples have shown that disease oligodendrocytes are highly affected in their transcriptome (Falcão et al. 2018; Jäkel et al. 2019; Schirmer et al. 2019; Trobisch et al. 2022; Macnair et al. 2023), with affected pathways including antigen presentation, inflammation, iron metabolism, and severe cell stress (Falcão et al. 2018; Jäkel et al. 2019; Schirmer et al. 2019). Based on dynamic changes in populations of oligodendrocytes, it has been suggested that fully mature, stable oligodendrocytes are lost in MS, in favor of oligodendrocytes with transcriptional programs that suggest they have alterations in their capacity for metabolic support, or inflammatory properties (Jäkel et al. 2019) (Figure 1). In particular, inflammatory OPCs (iOPCs) and oligodendrocytes (iOLs) expressed gene modules associated with interferon response and major histocompatibility complex (MHC)-I and -II genes in the EAE model for MS (Falcão et al. 2018), while in MS tissue, only iOLs were identified (Jäkel et al. 2019). IFNγ was shown to be a key for the induction of these gene modules in OPCs in vitro (Falcão et al. 2018). Through their expression of immune-related molecules, these iOPCs and iOLs are thought to contribute to disease progression in multiple ways. iOPCs have been shown to have the capacity to attract $CD8^+$ T-cells, resulting in a feedback loop that ultimately leads to their cell death (Kirby and Castelo-Branco 2021). iOPCs and iOLs have also been reported to be associated with the regulation of the extracellular matrix, which in turn affects remyelination capacity (Kirby and Castelo-Branco 2021). However, the underlying factors contributing to the observed differences in the states of oligodendrocyte lineage cells between mouse EAE models and human MS remain unclear. Interestingly, a study analyzing cells from distinct areas in MS donors, including spinal cord, cerebellum and leukocortical areas, identified a subtype of white matter oligodendrocytes in MS lesions that are predicted to engage in remyelination (Trobisch et al. 2022). With respect to MS lesion pathology, it is known that paramagnetic rim lesions are characterized by iron uptake and disturbed iron metabolism. Iron distribution strongly varies between normal-appearing white matter, where oligodendrocytes and myelin show the strongest iron staining, while at lesion rims, iron is concentrated in reactive astrocytes and activated myeloid cells (Popescu et al. 2017). Further, it has been shown that a reduction of iron in oligodendrocytes was linked to an increased expression of iron-exporting ferroxidases in those cells (Hametner et al. 2013).

Collectively, those findings indicate that oligodendrocytes are not purely the target of the immune response in MS, but that they also respond strongly to pathology and thereby contribute to both lesion progression and repair. However, the precise spatial patterns and functional properties of oligodendrocyte subtypes throughout MS lesions need to be elucidated in future studies. Applying in situ sequencing, a novel spatial transcriptomic method allowing for the detection of a few hundred transcripts, to the developing mouse CNS has provided insight in the spatial distribution of the different oligodendrocyte lineage cells in stages of development. It was shown that their progression through development is affected by space. Depending on the brain region, differentiation of oligodendrocytes and myelination proceeded at different rates with distinct types of oligodendrocytes preferentially neighboring each other. In addition, differences in dynamics were observed between gray and white matter regions in both spinal cord and brain (Hilscher et al. 2022). Moreover, another recent study utilizing in situ RNA sequencing (ISS) in mouse spinal cords allowed dissecting both spatial and temporal processes during experimental autoimmune encephalomyelitis (EAE), a classic MS-related model (Kukanja et al. 2024). The annotation of spatially restricted neighborhoods suggested a model of lesion evolution, in which active lesions develop and progress in a centrifugal fashion. Of note, it has been found that disease-associated (DA)-glia in EAE arose independently of lesions, and dynamically appeared and resolved over the disease course. This included subtypes of disease-associated microglia, astrocytes and oligodendrocytes (Kukanja et al. 2024).

Spatial transcriptomics of frozen human brain samples identified mechanisms of progressive MS pathogenesis in relation to spatially distributed stages of neurodegeneration in the gray matter (Kaufmann et al. 2022). Also changes in NAWM were detected by ST, with reported changes in microglia, oligodendrocytes and OPCs (Lam et al. 2023). The importance of space as a factor in white matter MS lesion development was also recognized by spatial transcriptomic analysis of human MS lesions (Alsema et al. 2024) (Lerma-Martin et al. 2024). Not only did ST identify a novel active lesion rim based on gene expression levels, the concept of centrifugal lesion progression was also suggested in human lesion development by computational analysis of lesion progression (Alsema et al. 2024). These studies furthermore highlighted the importance of oligodendrocytes and their altered gene expression programs and interactions in lesion typing and development.

The changes in oligodendrocytes are likely of high importance for disease progression, as suggested by several snRNA-seq studies in MS indicating that the potential for tissue repair seems to be encoded in the transcriptional response of oligodendrocytes. What has overall become clear is that oligodendrocytes are severely affected in MS, and that the damage to oligodendrocytes and myelin and the altered dynamics of myelin, are highly dependent on interactions with other cell types.

3 | Spatially Encoded Interactions Driven by Glial Cell Types

Though highlighted as independent cell types above, the cells in the CNS do not act as independent entities, rather they share extensive intercellular communication in order to both fulfill

homeostatic functions, but also to be able to respond to damage and disease. Multiple mechanisms are employed by cells to coordinate this communication, that involve interactions between secreted ligands and plasma membrane receptors, yet it also includes secretases, extracellular matrix proteins, microtubules, extracellular vesicles, transporters, and direct cell-to-cell contact mechanisms through receptors or for example gap junctions (Su et al. 2024). As protein interaction databases continue to expand, RNA sequencing data can be used to infer cell–cell interactions and communication, such as through ligand-receptor pair analysis (Armingol et al. 2021). In a recent postmortem MS tissue study, spatially-encoded ligand-receptor pairs between glial cells and immune as well as endothelial cells were predicted through computational algorithms based on paired snRNA-seq and spatial transcriptomics (Lerma-Martin et al. 2024) that demonstrate the power of omics data integration to predict cell–cell communication events in the context of MS. However, as most interactions in the context of neuroinflammation have been described for animal models, in particular, glial-endothelial, or blood communication events (Merlini et al. 2019; Mendiola et al. 2023), future studies need to put a stronger emphasis on validation of those interactions in human tissues.

3.1 | Glial–Immune Cell Interactions

With auto-immunity as a key component of MS pathophysiology, and the identification of glial cells at the borders of lesions as likely drivers of lesion initiation and progression (Figure 2), the interactions between immune cells and different glial cell types are at the core of MS progression. Though it is not clear whether autoimmunity is the sole cause of MS or a consequence of a maladaptive cytological process in the CNS, which subsequently triggers an immune response to myelin. What is clear is that peripheral T- and/or B-cells are activated and infiltrate the CNS via an apparently dysfunctional (BBB) (Sen et al. 2020). In active MS lesions, the majority of immune cells in the lesion consist of monocyte-derived macrophages and microglia, with more limited contribution of lymphocytes, mostly $CD8^+$ T cells and less pronounced $CD4^+$ T cells (Dendrou, Fugger, and Friese 2015; Ingelfinger et al. 2022). The cells infiltrating the CNS are producing a large array of cytokines that are sensed by and trigger resident glial cells with astrocytes and microglia showing the strongest responses (Becher, Spath, and Goverman 2017; Amoriello et al. 2024). Several such cytokines, including IL1B, TNFa, IL6, IL17, and IFNγ have been shown to activate other immune cells, orchestrate CNS trafficking and, through their actions on astrocytes and microglia, promote tissue damage and drive inflammation.

Typically, the number of tissue-infiltrating cells that are identified in sc/snRNA-seq studies from human MS samples is limited, given their relatively low abundance compared to the resident neuroglial cell types. For example, in white matter, generally > 80% of all cells are oligodendrocyte lineage cells. As a result, it has been more challenging to address the heterogeneity of tissue-infiltrating cells in CNS tissues and predict interactions based on currently available datasets. Further, the presence and distribution of immune cells highly depend on the inflammatory type of MS lesions, or rather, the lesion age. Active/inactive and inactive lesions show highly variable numbers of infiltrates that mainly localize to the lesion core and rims of active demyelinating and mixed active/inactive lesions. Therefore, to delineate the crosstalk between glial and immune cells and the effects on MS lesion fate will largely depend on the analysis of cell–cell communication events in time and space.

In human postmortem MS samples, the temporal component is difficult to capture, though computational methods can be employed to predict developmental trajectories of MS lesion development and progression (Alsema et al. 2024). Trajectory analysis uses algorithms to map the sequence of gene expression changes that each cell, or spatial location in the case of spatial transcriptomics, undergoes during a dynamic biological process. By learning the overarching "trajectory" of gene expression changes, this method arranges individual cells or spatial spots along the trajectory, revealing their (pseudo)temporal relationships. In the EAE mouse model, however, samples can be collected at different timepoints along lesion progression. Using ISS-based spatial gene expression analysis, it has been recently demonstrated that interaction events between immune and glial cells were highly dynamic and increased in EAE. These interactions occurred between disease-associated glia, T cells, and myeloid cell subtypes and appeared in close proximity to the vasculature and ependymal cells (Kukanja et al. 2024). The occurrence of disease-associated glia (including oligodendrocytes) at sites distant from infiltrates does not preclude a role for more distant signaling events between these cell types in the process. This is concordant with the reported induction of oligodendroglial states in MS through, for instance, interferon gamma (IFNγ) that can be produced by T cells (Olsson et al. 1990; Falcão et al. 2018; Kirby et al. 2019). During EAE progression, gene expression patterns and the degree of interactions between immune and glial cells dramatically changed. Disease associated-glial cells initially distributed in a localized manner within regions sparsely infiltrated by immune cells. Spatial dissection of lesion development over time in EAE has resulted in a model of neuroinflammatory lesion evolution that mainly depends on disease-associated glia in contact with surrounding immune cells, thereby, inducing subtype changes and contributing to the progression of EAE (Kukanja et al. 2024).

3.2 | Glial–Neuronal Interactions

Interactions between glial cell types and neurons are fundamental to maintain homeostatic function of the nervous system. Because of the complexity of MS lesion formation with a distribution of lesions in various gray and white matter areas, an understanding of the physiological and pathological communication events between glial and neuron subtypes with respect to the tissue environment is key. The strongest glia–neuron interaction is probably related to myelin ensheathment of nerve fibers. As demyelination and oligodendrocyte death are classical features of MS pathology, fiber wrapping is likely one the most important resilience factors in the nervous system and, specifically to MS, also the strongest vulnerability determinant. It has been shown that the sodium channel Nav1.6 and the sodium-calcium exchanger (NCX) can be found together with the axon damage marker β-amyloid precursor protein (β-APP) on demyelinated axons in MS (Craner et al. 2004; Black et al. 2007). Of note, those ion channels are

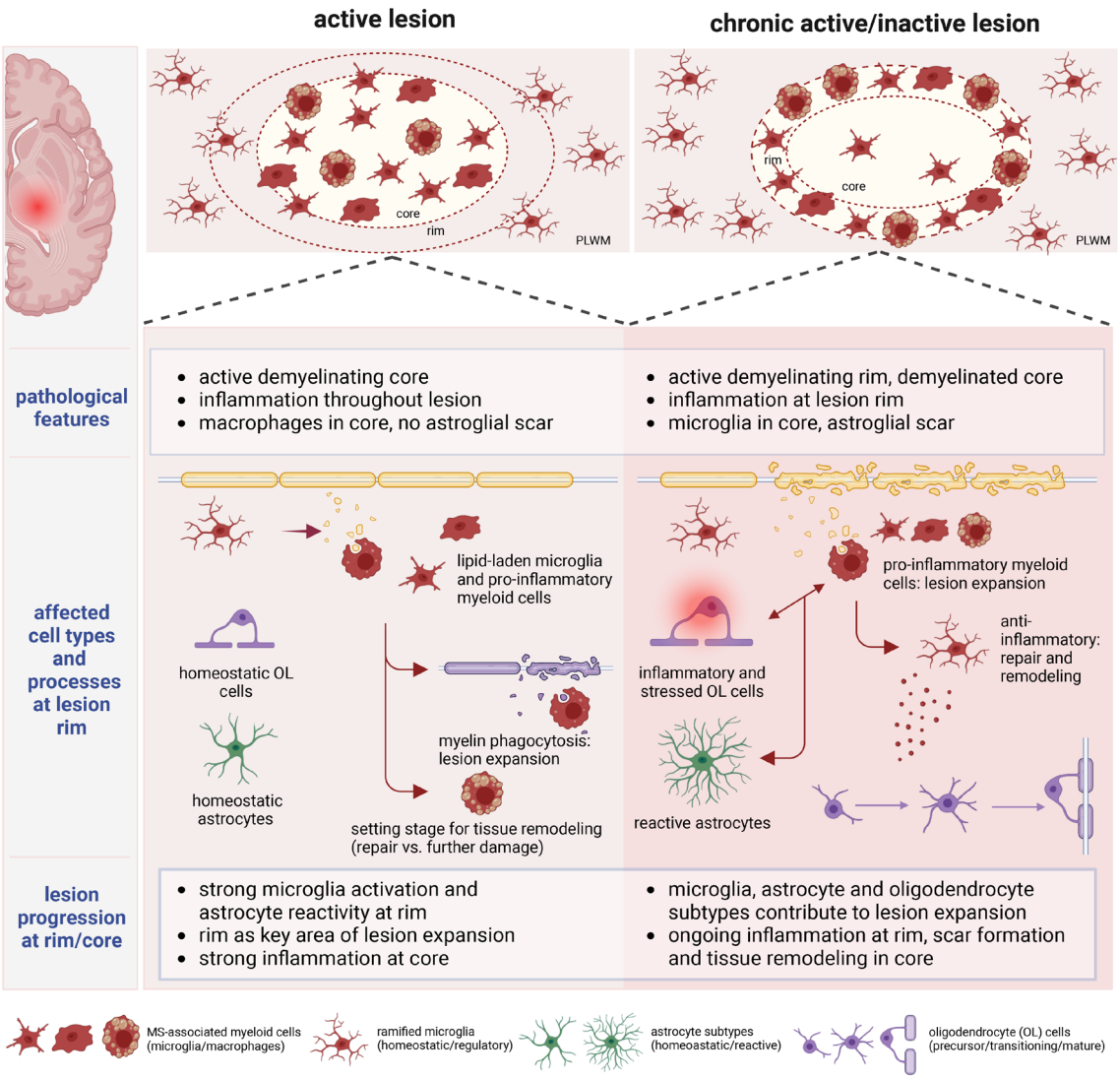

FIGURE 2 | MS lesion rim as critical hub for lesion progression. A schematic overview of active and mixed chronic active/inactive MS lesions and the cellular processes that are affected in those lesion types. Myeloid cells, including activated microglia and tissue-infiltrating macrophages, are the main effector immune cells present in active lesions with critical roles in myelin phagocytosis but also contributing to repair and tissue remodeling. Astrocytes represent a highly dynamic group of glial cells with distinct pro- and anti-inflammatory functions with reactive subtypes present at inflamed lesion rims and scar forming cells in chronic lesion cores. Conversely, oligodendrocytes are eventually lost in the course of MS lesion development and exhibit strong transcriptomic changes in the context of cellular stress and inflammatory response patterns in the proximity to activated myeloid cells and reactive astrocytes. Overall, the state and degree of pro- and anti-inflammatory glial cell types at the lesion rim is thought to play key roles in the long term damage and repair of MS lesions. Created with BioRender.com

usually not found on axons under physiological conditions and can alter neuronal excitability and increase the risk to degenerate in situations such as in MS. Likewise, destabilization and loss of paranodal structures that are formed by oligodendrocytes and myelin scaffolds are risk factors in MS pathology and are associated with axon damage (Coman et al. 2006; Howell et al. 2010).

Previous work found that oligodendrocytes and astrocytes lose some of their functions related to potassium uptake that build up during neuronal excitability and saltatory conduction. Specifically, loss of potassium uptake function was shown for the inward-rectifying potassium channel KIR4.1 that was lost on both astrocytes and oligodendrocytes in subcortical white matter lesions (Schirmer et al. 2014). Related work in animal models of inflammatory demyelination could then demonstrate that Kir4.1 loss of function results in neuronal hyperexcitability, increased levels of tissue damage and, eventually, clinical worsening and increased mortality of animals during aging (Schirmer et al. 2018) and progressive neuroinflammation (Kapell et al. 2023). Notably, in the latter study the small molecule retigabine was applied, which activates nodal Kv7 channels along axons that could prevent some of the damage caused by lost Kir4.1 functions. Hence, this work highlights the importance of glial-neuronal signaling as a neuroprotective resilience factor relevant under inflammatory demyelination. Overall, those studies suggest that glial cell types lose important homeostatic and neuron-supporting functions during neuroinflammation and adopt maladaptive functions that can trigger damage and subsequent death of neurons. Other work focused

on astrocyte-microglia signaling and found that activated microglia upregulated axon guidance cues such as semaphorin 4D and ephrin-B3, which were able to convert homeostatic astrocytes into a proinflammatory phenotype. Astrocyte-microglia crosstalk is also critical for synapse maturation and maintenance as, for example, shown for IL-33 signaling (Vainchtein et al. 2018). Of note, increased IL-33 levels were found in the serum of MS patients (Mado et al. 2021); however, future work is needed to demonstrate the role of IL-33 in MS and related models.

Another example of maladaptive neuron-astrocyte dysfunction is related to A1-polarized astrocytes that were shown to secrete saturated fatty acids that exhibit neurotoxic properties (Guttenplan et al. 2021). In MS, damage to the anterior visual pathways is common and can be nicely studied in patients through retinal optical coherence tomography. Regarding complement factors and a potential A1 astrocyte subtype reactivity in MS, it could be shown that loss of complement factor 3 (C3) function in astrocytes could protect neurons and delay neurodegeneration along the anterior visual pathways in experimental autoimmune encephalomyelitis (Werneburg et al. 2020). As astrocytes and microglia are key players in the maintenance of neuronal circuitry and synapse function, future work related to MS is needed to make connections between disturbed and maladaptive glial cell functioning and neuronal dysfunction.

4 | Challenges and Perspectives in Glial Cell Type Omics

Recent technological developments have dramatically advanced our understanding of the molecular tissue composition and its changes in MS pathology. The introduction of cell type-specific transcriptomics has been a key in characterizing and precisely defining glial cell types and their subtypes based on gene expression (Figure 1). However, so far most of these studies have been applied to cryopreserved human postmortem tissues derived from deceased individuals with MS (Jäkel et al. 2019; Schirmer et al. 2019; Absinta et al. 2021; Trobisch et al. 2022; Seeker et al. 2023; Kukanja et al. 2024). This means that the results reflect, more or less, the tissue composition of a long-standing disease and, therefore, have limited value for the identification of molecular disease signatures linked to the onset of the disease. A further consequence of this is that the currently available data lacks the capacity to evaluate whether intrinsic differences drive the heterogeneity in symptoms and clinical progression associated with relapsing–remitting and progressive disease courses. Hence, there is a high clinical and translational need to explore molecular and, ideally, cell type-specific dynamics in tissue samples reflecting early pathological changes in MS. Rarely, the diagnosis of MS is settled based on histopathological assessment of biopsies; however, such tissue samples are available and it is likely that future omics studies will include such samples in research. The same holds true for human brain tissue in general, where freshly processed biopsy tissues and archival biopsy tissues of short storage interval are favored over postmortem tissues. Indeed, some recent studies have included fresh biopsy (Masuda et al. 2019; Sankowski et al. 2024) and fresh autopsy tissues (Wheeler et al. 2019, 2020; Miedema et al. 2022) for analysis of transcriptomics changes in glial cell types. The potential use of biopsied tissue is further aided by technological developments that now enable the use of (archived) formalin-fixed paraffin-embedded tissue in several omics approaches, including spatial transcriptomics.

The overall temporal progression of MS lesions is poorly captured in human tissue samples, whether postmortem or biopsied. Animal models of the disease, such as EAE and toxic models of de- and remyelination, can provide valuable insights into the temporal and spatial dynamics of lesion progression (Kukanja et al. 2024). However, none of the current animal models fully replicate the complexity of MS. Notable differences between MS and its animal models have been observed, particularly in glial cell diversity. For instance, iOPCs are present in EAE but have not been identified in human MS samples (Falcão et al. 2018; Jäkel et al. 2019; Schirmer et al. 2019). This discrepancy could be due to differences in the inflammatory lesion stage at the time of analysis or may reflect intrinsic inter-species differences.

In addition, future work with postmortem tissues should put emphasis on careful stratification of different MS tissue subgroups. For example, one idea would be to dissect and annotate particular anatomical regions or well-defined lesion and nonlesion areas to reduce sampling bias driven by anatomical differences in tissue and cell type composition. Also, subgroup stratification could be further enhanced through categorizing tissues based on disease duration or the immune therapy administered during lifetime. Independent from tissue sampling, the most critical challenge is identification and selection of tissue samples suitable for multi-omic technologies that should include quality control measurements such as the RNA integrity. Other factors with critical impacts on the quality of gene expression studies are postmortem intervals for autopsy and freeze/fix intervals post sampling for biopsy studies, respectively.

Another current challenge in glial cell type research in MS is the lack of spatially resolved data about glial subtypes between normal-appearing and MS lesion areas, including the lesion rim and the core. Spatial transcriptomics and highly multiplexed in situ RNA hybridization assays are one way to precisely characterize cell type-specific gene expression in a spatial context; however, they are limited due to a lack of a high (cell- to subcellular) resolution or issues with cell segmentation in case subcellular resolution is achieved. A recent study has successfully applied in situ sequencing at high resolution to map glial cell type changes in MS spinal cord tissues (Kukanja et al. 2024). Other emerging technologies such as multiplexed immunohistochemistry using either antibodies tagged with fluorescent or metal compounds such as in imaging mass cytometry are another way to characterize glial cell type and have the advantage of imaging at a high optical resolution. Of note, a recent study on COVID19 has applied imaging mass cytometry to analyze glial cell type diversity at high resolution (Schwabenland et al. 2021). Additional tools such as high-resolution imaging of epigenetic processes using, for example, spatially resolved CUT&Tag assays (Deng et al. 2022; Bartosovic and Castelo-Branco 2023) will help better understand the regulatory mechanisms related to glial subtypes in the context of MS. To acquire high-resolution images is a critical issue, particularly, in MS research, as rather small areas such as the lesion rim associated with defined glial subtypes have important roles in the propagation of

lesion development and, at a larger scale, in disease progression (Figure 2).

Downstream of sampling and imaging are data-driven challenges that relate to the assessment and computational prediction of tissue niche composition and cell–cell communication events. For example, algorithms such as BayesSpace have been developed that allow a precise segmentation of tissue niches such as cortical gray matter layers (Zhao et al. 2021). Another emerging topic is about new methods to calculate interactions between glial cells and neighboring neurons and immune cells based on ligand-receptor pairs in tissue niches. Therefore, multicellular factor analysis tools have been developed that allow mapping of cell types and calculating communication events in a spatial context (Argelaguet et al. 2020; Dimitrov et al. 2022; Ramirez Flores et al. 2023), which have been successfully applied to diseases like myocardial infarction (Kuppe et al. 2022) and MS (Lerma-Martin et al. 2024). Recent computational advances have helped better predict such cell–cell communication events, for example, using deconvolution algorithms such as cell2location that allow integration of both single-cell and spatial gene expression data (Kleshchevnikov et al. 2022; Li et al. 2023) and were recently validated in MS tissues (Lerma-Martin et al. 2024). Finally, to assess the temporal component in lesion development and disease progression in MS will remain a challenge. Predictions can be made using computational approaches (Alsema et al. 2024), and certain lesions can be tracked over time by MRI (Absinta et al. 2021). Given the inability to sample at multiple timepoints, future analyses in this area will depend on improved computational methods, improved sensitivity in omics approaches and improved methods for imaging lesions that, for example, include quantitative measurements of myelin (de Paula 2020; Absinta et al. 2021) to track demyelination and myelin repair.

Single-cell/nucleus and spatial transcriptomics have significantly advanced our understanding of the cellular heterogeneity driving the diversity of lesion pathology and progression in MS. These approaches have not only identified new biomarkers but also provided valuable insights into pathways that could be targeted for therapeutic intervention. The discovery of distinct glial cell populations in specific lesion areas corresponding to particular inflammatory stages suggests the existence of critical windows for glia-focused therapeutic interventions, which future studies must precisely define. Furthermore, recent studies utilizing spatially resolved transcriptomics have highlighted the rim as a crucial area in MS lesion progression, uncovering distinct cellular interactions, and gene expression changes that drive lesion dynamics. These insights provide valuable biomarkers and potential therapeutic targets, offering a foundation for precision therapies aimed at modifying lesion development in MS.

5 | Concluding Remarks

Recent years have dramatically changed our view about the multifaceted roles glial cells play in chronic neuroinflammation. In that regard, MS remains the prototypic disease to study spatial as well as temporal patterns related to glial subtype diversity and compositional changes related to specific tissue niches during lesion development. In this review, we have highlighted the diversity of glial subtypes and their specific responses relevant to MS pathology. Further, we have put an emphasis on the latest technological developments and the need to develop approaches for a precise monitoring and therapeutic modulation of specific glial subtypes in time and space.

Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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