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Astrocyte signaling and interactions in Multiple Sclerosis

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Abstract

Multiple Sclerosis (MS) is a common cause of impairment in working-aged adults. MS is characterized by neuroinflammation and infiltration of peripheral immune cells to the brain, which cause myelin loss and death of oligodendrocytes and neurons. Many studies on MS have focused on the peripheral immune sources of demyelination and repair. However, recent studies revealed that a glial cell type, the astrocytes, undergo robust morphological and transcriptomic changes that contribute significantly to demyelination and myelin repair. Here, we discuss recent findings elucidating signaling modalities that astrocytes acquire or lose in MS and how these changes alter the interactions of astrocytes with other nervous system cell types.

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Introduction

MS is a chronic inflammatory and degenerative disease that clinically presents with demyelinating plaques in the Central Nervous System (CNS). Demyelination damage causes MS patients to have motor impairments, muscle weakness, and cognitive dysfunction, all leading to poor quality of life [1]. MS has multifactorial etiology, including genetic, infectious, and environmental predispositions [2]. Regardless of the trigger, lymphocytes and myeloid cells become activated and then generate autoantibodies against proteins expressed in the myelin and the oligodendrocytes, the myelinating cells of the CNS [3]. The specific role of these autoantibodies is still debated; however, it is thought that CNS cells are exposed to autoantibodies once lymphocytes cross the blood–brain barrier (BBB) [4]. This inflammatory environment causes degeneration of multiple neural cell types and components, such as myelin, axons, synapses, oligodendrocytes, and neurons [5,3].

Astrocytes, the most abundant glial cell type in the CNS, are critical for the health and function of CNS [6]. In particular, astrocytes promote oligodendrocyte development and myelination by secretion of lipoproteins and growth factors and by forming gap junctions with oligodendrocytes [7]. Extensive evidence indicates that astrocytes, gain a reactive phenotype in MS [8]. Due to the emerging roles of astrocytes as critical regulators of CNS homeostasis and pathology, these cells recently became a central topic for investigation in MS. This review discusses literature from the past ten years, with a particular focus on how astrocytes lose homeostatic functions or gain pathological responses during MS. We will specifically highlight the inter- (secreted) and intra-cellular signaling pathways these glial cells were shown to use during the different stages of the disease.

Astrocytes lose homeostatic functions and gain neuroinflammatory phenotypes in MS

Evidence acquired over the last decade indicates that astrocytes display multiple cellular states in MS, which vary by neuroanatomical region and proximity to the demyelinating plaques [9,10]. Studies using *postmortem* human brain tissue and single nuclei (sn)-RNA-seq revealed that the genetic profile of astrocytes from cortical gray matter (GM) and subcortical white matter (WM) differs significantly [10]. Moreover, proximity to chronically active or inactive MS lesions or the location of cells with regards to the lesion, such as peri-plaque, edge, and core, impacted the astrocyte gene expression. These changes ranged from losing homeostatic functions of astrocytes to gaining neuroinflammatory properties which we will discuss further below [9–12].

Astrocytes give crucial support to oligodendrocytes by providing lipids such as cholesterol which are critical to maintain myelin integrity (reviewed in Ref. [13]), a

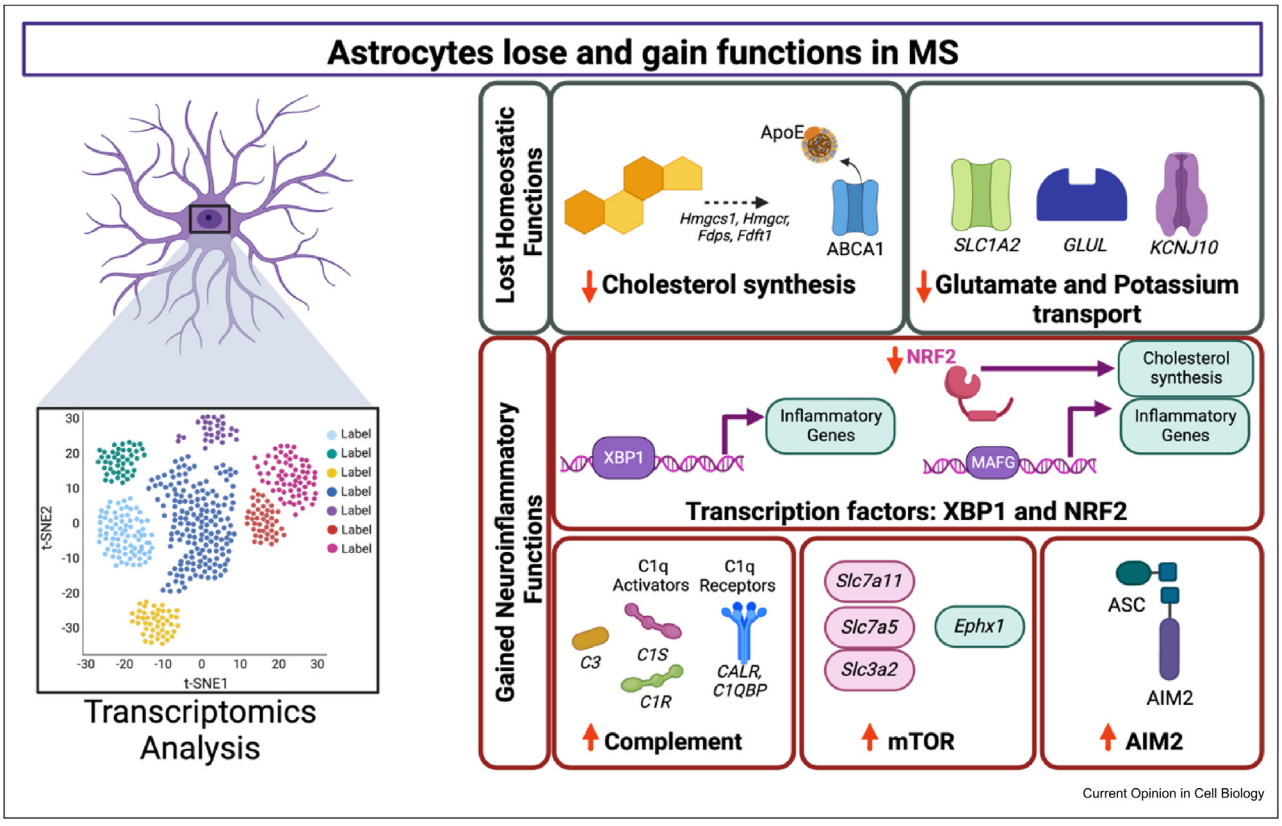
pivotal astrocytic function perturbed in MS (Figure 1). In a commonly used mouse model of MS, the experimental autoimmune encephalomyelitis (EAE), Itoh and others discovered a region-specific decrease in astrocytic mRNA expression of genes associated with cholesterol biosynthesis, using the RiboTag technology, which determines the cell-specific active translatome. This decrease in cholesterol synthesis genes was specific to the astrocytes from the spinal cord, cerebellum, and optic nerve at the peak of demyelination. Providing an agonist for the cholesterol transporter ABCA1, which increases extracellular cholesterol associated with ApoE particles, decreased EAE disease severity [12]. A decrease in cholesterol synthesis has also been identified in the WM of *postmortem* MS brain tissues [14]. These results indicate that loss of cholesterol signaling by astrocytes is a critical pathological mechanism in MS.

Astrocytes also tightly regulate extracellular neurotransmitter and potassium ion concentrations to maintain proper synaptic transmission [15]. Another study, using sn-RNA-seq analyses of the cortical WM and GM *postmortem* human brain tissues, identified a GM astrocyte subpopulation in MS patients with severely downregulated expression of glutamate transporter *SLC1A2*, glutamine synthetase *GLUL*, and potassium channel *KCNJ10* [10]. These findings suggest that in MS, astrocytes lose their homeostatic functions that regulate glutamate and potassium ions. This dysfunction can lead to excitotoxicity and neuronal death seen in MS.

On the other hand, astrocytes were also shown to secrete molecules that exacerbate inflammation in MS, which limits recovery [16] (Figure 1). Sn-RNA-seq data from the WM *postmortem* human MS brains revealed five different classes of astrocytes within active demyelinating plaques: (1) non-reactive, (2) reactive/stressed, (3) Astrocytes Inflamed in MS (AIMS), (4) senescent, and (5) perinodal. Of these classes, the AIMS group upregulated the expression of reactivity genes such as *GFAP*, *APOE*, *VIM*, *S100B*, *SOD1*, and *Complement 3* (C3) [9].

Other relevant neuroinflammatory gene expression changes in astrocytes in MS included (1) mammalian

Figure 1



Astrocytes lose and gain functions in MS. Some recent transcriptomic analysis from mouse models and *postmortem* tissue revealed that astrocytes lose and gain functions in demyelination. In MS, astrocytes lose seminal homeostatic functions such as cholesterol biosynthesis [12,39,14] and glutamate and potassium transport [10]. Concurrently, astrocytes gain neuroinflammatory functions that affect their crosstalk with oligodendrocytes, microglia, and neurons. Some of these neuroinflammatory functions are acquired through the modulation of transcription factors such as XBP1 and NRF2 [18,19,22]. In turn, they activate the expression of inflammatory genes and pathways like complement [9], mTOR [17], and AIM2 [20].

Target Of Rapamycin (mTOR), (2) X-box Binding Protein 1 (XBP1), (3) nuclear factor erythroid 2—related factor 2 (Nrf2), and (4) activation of the inflammasome Absent in Melanoma-2 (AIM2) (Figure 1) [17—20]. We will discuss these changes and their proposed signaling mechanisms next.

A recent study used the cuprizone mouse model of demyelination to profile cellular responses to myelin loss and repair. sn-RNA-seq of the cells from the demyelinated corpus callosum at the peak of disease revealed that astrocytes increase the expression of genes related to the mTOR pathway that promotes cellular growth and suppresses autophagy [17]. Interestingly, inhibitors of the mTOR pathway have been clinically tested and shown to reduce MS active lesions [21]. However, whether astrocytes are the cellular targets of these pharmaceuticals has yet to be studied.

The expression of the transcription factor XBP1 characterizes another subclass of astrocytes in MS. XBP1 was initially identified by Wheeler and others in a zebrafish demyelination model (i.e., combined cuprizone and lipopolysaccharide (LPS) treatment) followed by a screen for 75 environmental toxins [18]. One of these toxins, the herbicide linuron caused the highest activation of pro-inflammatory cytokines in murine astrocytes *in vitro*. Upon further analysis, this study found that astrocytic exposure to linuron mimics the effects of pro-inflammatory cytokines IL-1β and TNF-α leading to a significant increase in XBP1 and a highly reactive phenotype. CRISPR/Cas9-mediated deletion of *Xbp1* in mouse astrocytes ameliorated EAE symptomatology. Astrocytes from WM and GM *postmortem* MS tissue also had increased XBP1 expression. A follow-up study, using nucleic acid cytometry, found that XBP1 activation leads to mineralocorticoid nuclear receptor (NR3C2) down-regulation, which limits the anti-inflammatory function of NR3C2 [18,22]. These findings show that astrocyte reactivity induced by immune and environmental insults can serve as molecular targets to limit neurodegeneration in MS.

Another mechanism by which astrocytes acquire neuroinflammatory properties is through a reduction in the transcription factor NRF2. NRF2 activity can be upregulated in astrocytes by deleting CSF2RB, a receptor for the pro-inflammatory cytokine GM-CSF. This manipulation reduces inflammation and ameliorates EAE phenotypes. Decreased expression of NRF2 was also found in WM astrocytes of MS *postmortem* tissue [19]. These studies suggest that MS triggers astrocytic transcriptional modifications that lead to the acquisition of different neuroinflammatory roles that promote disease.

AIM2 mediates another inflammatory pathway in astrocytes relevant to the EAE model. Even though AIM2 is a protein localized to the inflammasomes, this pathway may have protective roles in MS. Inflammasomes are specialized multi-protein complexes formed following the activation of pattern recognition receptors. Assembly of inflammasomes triggers caspase-1 activation that induces cytokine release and inflammation [23]. Barclay and others found that loss of AIM2 in mice exacerbates EAE phenotype and alters microglial and astrocytic reactivity. The authors then utilized a transgenic mouse that labels inflammasomes with a florescent protein (Adaptor apoptosis-associated Speck-like protein Containing a CARD (ASC) conjugated to citrine). They found that inflammasomes are highly prevalent in the astrocytes from the spinal cords of the EAE mice. The astrocytic inflammasome assembly was not linked to astrocyte death or the release of inflammatory cytokines [20]. This study did not confirm the expression of AIM2 in MS human tissue. However, it revealed that not all neuroinflammation-associated astrocytic proteins cause pathologic effects.

In summary, these studies indicate that astrocytes lose homeostatic functions and gain neuroinflammatory responses in MS, pinpointing that specific therapeutic targeting of astrocyte subtypes could limit the progression of MS. However, there is a need for proof-of-concept studies and toxicity implications of targeting astrocytes in MS. In the following sections, we will delve into the molecular and cellular mechanisms underlying the astrocytic cell—cell interactions in MS.

Astrocyte-oligodendrocyte communication is impaired in MS

Astrocyte-secreted factors are crucial for oligodendrocyte survival, proliferation, and migration [24—27]. Indeed, if astrocytes are ablated, oligodendrocyte precursor cells (OPCs) cannot differentiate, mature, and myelinate axons [28,29]. Astrocytic gap junction coupling to oligodendrocytes through connexins is also imperative for proper myelination [30]. Expression of connexins is significantly altered in MS [31], and when connexins 32 and 47 are knocked out in mice, EAE pathology worsens [32], underscoring the importance of astrocyte-oligodendrocyte coupling in myelin health.

Astrocytes secrete glial-cell-derived neurotrophic factor (GDNF), which signals to OPCs to promote proliferation [33]. In the spinal cord and cerebellum of EAE mice, GDNF secretion by astrocytes might be impaired. Jin and others found SARM1, an adaptor protein for innate immune responses, to be increased significantly in the astrocytes of these regions after demyelination. Genetic ablation of astrocytic SARM1 resulted in a delay of EAE onset and protected neurons. Mechanistically, they found that increased levels of SARM1 down-regulate astrocytic GDNF secretion, worsening demyelination, and inflammation [34]. These finding reveals that in EAE, astrocytes decrease the production of

growth factors, leading to impaired OPC proliferation and oligodendrocyte differentiation. Therefore, stimulation of growth factor secretion by astrocytes may benefit myelin repair, which should be assessed further in human studies.

As discussed earlier, in MS, some astrocytes lose the ability to synthesize cholesterol. Cholesterol is a principal component of myelin [35,36], and during adulthood, astrocytes are the primary cholesterol producers in the CNS [37,38]. Loss of cholesterol secretion could contribute to demyelination by interfering with oligodendrocyte and myelin homeostasis. On the other hand, demyelination may be the trigger for loss of cholesterol secretion from astrocytes. The impaired cholesterol synthesis in reactive astrocytes [39,12] would limit the capacity of oligodendrocytes to repair myelin.

The latter possibility, how deficient cholesterol biosynthesis in astrocytes affects remyelination, was studied by Molina-Gonzalez and colleagues in the myelin toxin Lysolecithin (LPC) demyelinating model. Using translating ribosome activity purification (TRAP), proteomics, and mechanistic studies, they determined that the downregulation of astrocytic NRF2 stimulates astrocytic cholesterol-synthesizing enzymes. Constitutive NRF2 overexpression in $GFAP^{+}$ astrocytes decreased cholesterol biosynthesis enzymes (HMGCS1, FDPS, MVD, and FDFT1). The number of oligodendrocytes decreased, and remyelination was impaired. They could rescue these phenotypes using a specific agonist of the cholesterol transporter, ABCA1 [14]. Taken together with other studies linking NRF2 to neuroinflammation in MS, these findings accentuate the importance of this transcription factor in astrocytes during the demyelination and remyelination stages of the disease (Figure 1) [19].

It is important to note that another study conditionally ablated squalene synthase (the first enzyme in cholesterol biosynthesis) using astrocyte-specific, Aldh1L1 promoter-driven, Cre recombinase expression and found that this genetic manipulation did not interfere with remyelination nor the number of mature oligodendrocytes in the cuprizone mouse model. However, the Aldh1L1 promoter is also active in the liver. Therefore, the mice supplemented with cholesterol to compensate for the loss of this enzyme in the liver [39], which could have affected these results. Further studies are needed to fully elucidate the functions of astrocytic cholesterol synthesis in oligodendrocyte survival and remyelination.

When astrocyte cholesterol synthesis is impaired, such as in the cuprizone-induced demyelination model, it is hypothesized that neurons start to produce cholesterol [40] and microglia generate desmosterol [39]—both important for remyelination and shown to be present in sequencing data of human MS [39,40]. This cholesterol precursor stimulates liver cholesterol synthesis and oligodendrocyte production of myelin [39]. These studies suggest that neurons and microglia can sense and compensate for the loss of astrocytic cholesterol synthesis. Moreover, these findings indicate that stimulating cholesterol synthesis across multiple cell types can promote remyelination and oligodendrocyte survival.

In MS, demyelination is often followed by remyelination; however, as the disease progresses, the efficiency of remyelination is diminished. Orthmann-Murphy and others, using the cuprizone model, showed that even though oligodendrogenesis occurs post-demyelination, the patterns and density of oligodendrocytes and myelination in the cortex do not return to the preinjury levels [28]. The attenuated remyelination was accompanied by sustained astrocyte reactivity (increased GFAP expression) [28], suggesting continuous maladaptive crosstalk between these glial cells. There might be a delicate balance of astrocytic functions in myelin debris clearance and myelin homeostasis after demyelinating injury, which may irreversibly affect the astrocyte-oligodendrocyte crosstalk [41]. Future studies investigating astrocytes' role in oligodendrocyte function and dysfunction are needed to address this important knowledge gap.

Aberrant astrocyte-microglia crosstalk during demyelination fuels neuroinflammation and causes synapse loss

Astrocytes and microglia regulate fundamental processes that keep neuronal homeostasis [42]. Both phagocytose synapses, degenerating axons and myelin, after injury or in disease [41,43—46]. Active signaling between these two cell types is proposed to regulate each other's function. Here, we will focus on microglial signaling to astrocytes in MS.

Microglia signal to astrocytes during MS through secretion of axon-guidance cues or pro-inflammatory cytokines. The former was identified by Clark and others in the EAE model using the rabies barcoded interaction followed by sequencing (RABID-seq) technique. The reactive microglia upregulated the expression of genes that encode axon guidance cues, Semaphorin 4D and Ephrin-B3 in EAE and similarly found in the WM *postmortem* MS brains. When astrocytes were treated with these factors *in vitro,* they released inflammatory cytokines. Blocking the transcellular receptor-ligand signaling between the microglial Ephrin-B3 and astrocytic EphB3 ameliorated EAE disease symptoms [47], revealing that maladaptively microglia and astrocytes communication leads to disease progression.

Under injury or insult, microglia release pro-inflammatory cytokines, which drive astrocytes into an inflammatory neurotoxic state. For example, activated microglial IL-1, TNF, and C1q, robustly changed astrocytic gene expression and resulted in the loss of homeostatic astrocyte functions [48,9]. Moreover, these reactive astrocytes secrete saturated fatty acids that are neurotoxic [49]. An important molecular signature of the neurotoxic astrocytes is the increased complement protein C3 expression.

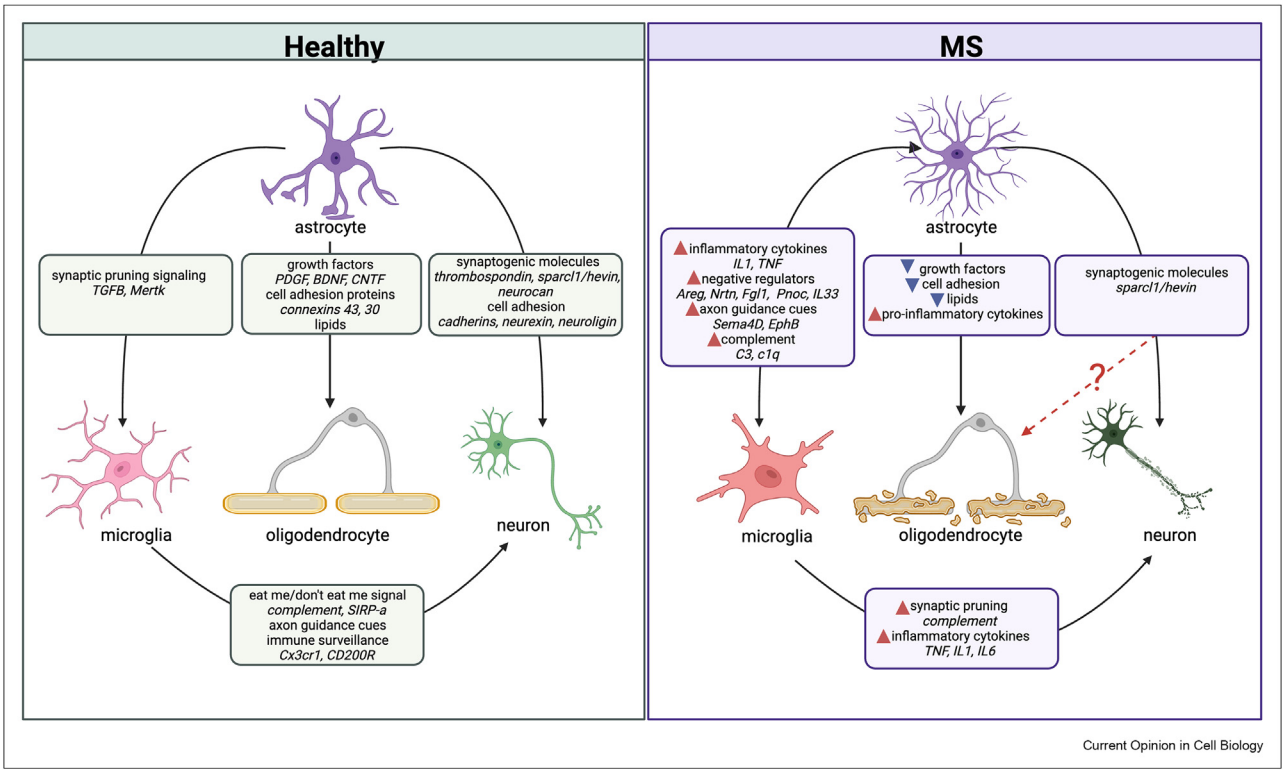
During development, the complement pathway regulates the pruning of synapses by microglial phagocytosis [50,51]. For example, weak synapses are tagged by the complement protein C1q in the retinogeniculate nucleus, which recruits C3 protein to these sites. C3 is processed into C3a and b fragments and recognized by microglia for phagocytosis. While some neurons and microglia express C1q, C3 in a diseased brain comes from reactive astrocytes [9,48].

The study by Absinta and others showed that, like astrocytes, microglia also display highly heterogeneous gene expression in MS, which varies by neuroanatomical region and the proximity to the demyelinating lesions. Using scRNA-seq from patient brains, they identified subclasses of microglia in an active demyelinating plaque, including Microglia Inflamed in MS (MIMS) near the AIMS astrocytes [9]. Their analyses revealed that AIMS upregulate C3 expression whereas MIMS increase the production of complement activators, including C1q and C3 receptors [9]. These findings suggest that AIMS-MIMS crosstalk via complement cascade stimulates pathogenic phenotypes.

Indeed, complement activation is known to trigger aberrant synapse pruning in MS. Werneburg and others found that in the EAE model of demyelination, C3 is recruited to retinogeniculate synapses, which are then phagocytosed by microglia through the C3 receptor. Inhibition of C3 protected synapse loss and rescued visual acuity in the EAE model [52]. Moreover, loss of astrocytic C3 expression protected retinal ganglion cells from death in the EAE model [53]. These studies suggest that in MS, microglia and astrocytes acquire maladaptive responses that affect neuron and synapse health through the complement cascade.

Microglial signaling may also limit maladaptive astrocytic responses in MS. Wheeler and others used a forward genetic screen via CRISPR/Cas9 targeting microglia and identified that microglial-secreted

Figure 2



Astrocytes participate in active crosstalk with microglia, oligodendrocytes, and neurons in health and MS. In health, astrocytes secrete factors and express proteins necessary for oligodendrocytes, microglia, and neuronal maintenance and survival. In disease, astrocytes acquire maladaptive responses that compromise the function of neurons, microglia, and oligodendrocytes while impeding myelin and neuronal regeneration. Similarly, astrocytes receive signals from microglia that prime astrocytes into proinflammatory and damaging phenotypes.

amphiregulin, a growth factor, signals to astrocytes through the epidermal growth factor receptor (EGFR). Both amphiregulin and EGFR increased in the WM of *postmortem* MS brains. Enhancement of amphiregulin/EGFR signaling protected the mice from EAE-induced demyelination [54]. Future studies should investigate if the cellular origins of IL33 are important for its protective roles in MS. Moreover, it would be fruitful to determine if microglia-astrocyte signaling via the amphiregulin-EGFR pathway prevents synapse loss and helps maintain neuronal function.

In summary, these studies show that maladaptive microglia to astrocyte signaling is a major mechanism underlying neuroinflammation in MS. In particular, complement activation is a significant mechanism of astrocyte-microglia communication in MS that leads to synapse loss.

Astrocyte dysfunction in MS leads to synapse loss and neurodegeneration

Astrocytes play key roles in the establishment and maintenance of neuronal circuits. They do so by physically ensheathing a majority of CNS synapses and by secreting synapse-modulating proteins and neuroactive molecules [55]. Moreover, astrocytes secrete molecules that trigger neuronal synapse formation and maturation and establish adhesions with synapses, which are critical for the maintenance of excitation and inhibition balance in healthy brains. Whether these mechanisms are altered in MS is not clear.

Sparcl1/hevin is an astrocyte-secreted molecule that controls the formation and plasticity of excitatory synapses by bridging presynaptic neurexin-1 (Nrxn1) and postsynaptic neuroligin-1 (Nlgn1) across the synaptic cleft [56]. Sparcl1/hevin expression was increased after cuprizone-induced demyelination in the mouse visual cortex [57]. However, another study found a significant reduction in *Sparcl1*/hevin mRNA at the peak of demyelination in the spinal cord of the EAE model mice [58]. Interestingly, a mutation in *NRXN1* was identified in a case study of a relapsing-remitting MS patient with no family history of MS [59]. As astrocytes undergo major gene expression and morphology changes during demyelination, astrocytes' roles at the synapse will likely be affected in MS. Future mechanistic studies investigating the impact of neuroinflammation induced by MS on the synaptic functions of astrocytes are needed.

Astrocytic signaling is also essential to support neuronal survival. A study by Kerkering and others demonstrated that a specific astrocyte-to-neuron signaling pathway protected neurons in benign MS, a form of relapsing-remitting MS characterized by mild attacks and long asymptomatic periods. Using patient iPSCs-derived astrocyte-neuron co-cultures, they found that benign MS patient astrocytes rescued neuronal damage induced by inflammatory cytokines [60]. On the other hand, astrocytes may also trigger neuronal death in MS. For example, in MS, neurons release ligands for the astrocytic growth factor receptor FGFR [61]. FGFR regulates astrocyte morphology [62] and is modulated by pro-inflammatory astrocytic responses [63], which can lead to neurodegeneration. This study by Kaufmann and others suggests that early changes in FGF signaling between astrocytes and neurons could contribute to MS pathology [61]. These studies provide evidence that there are many modes of astrocyte-neuron signaling in MS. Therefore, future studies are needed to identify them and determine their validity as targets for MS therapy.

Table 1

Pertinent questions of interest to address in future studies.

Unanswered questions for future studies.

General Topic	Specific Question
Molecular mechanisms	1. What molecular mechanisms underlie the robust and distinct transcriptional changes astrocytes undergo in MS?
	2. What are the molecules and signaling pathways that maintain myelination patterns in healthy aging, and how are these pathways affected in MS?
Cellular populations	3. Why do some astrocyte populations lose their functions, whereas others gain inflammatory ones?
	4. Do the maladaptive changes in astrocytes target specific neuronal populations, why?
Myelin integrity	5. How do astrocytes maintain a balanced cholesterol biosynthesis to ensure myelin integrity?
Synapse biology	6. Are the synaptic functions of astrocytes impaired across the course of MS?
Translational medicine	7. Can we identify protective astrocytic responses to MS and use them to device new therapeutic strategies for MS?

Conclusions

In MS, astrocytes undergo transcriptional and morphological changes, which result in the loss of critical homeostatic functions. Concomitantly, astrocytes gain neuroinflammatory roles through interactions with microglia and signals from the inflammatory environment in MS. These pathological characteristics compromise oligodendrocyte and synapse health (Figure 2). The findings discussed in this review stress the importance of studying astrocytes as a major regulator of demyelination and remyelination. Understanding the complex responses of astrocytes during demyelination and remyelination across different

neuroanatomical regions is critical to finding ways to target pathologies associated with MS. Thus, future studies, and efforts should focus on understanding key knowledge gaps in our understanding and exploring the translational potential of astrocytes in MS (Table 1).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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