The Role of Astrocytes in Multiple Sclerosis

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The role traditionally assigned to astrocytes in the pathogenesis of multiple sclerosis (MS) lesions has been the formation of the glial scar once inflammation has subsided. Astrocytes are now recognized to be early and highly active players during lesion formation and key for providing peripheral immune cells access to the central nervous system. Here, we review the role of astrocytes in the formation and evolution of MS lesions, including the recently described functional polarization of astrocytes, discuss prototypical pathways for astrocyte activation, and summarize mechanisms by which MS treatments affect astrocyte function.

Keywords: astrocytes, multiple sclerosis, neuroinflammation, NF-κB, risk variant, leukocyte recruitment

INTRODUCTION

Multiple sclerosis (MS) is an autoimmune disease that targets the central nervous system (CNS) (1). It is the most common, non-traumatic neurological disorder in young patients and affects nearly 1 million people in the US alone (2). In the majority of MS patients, the disease manifests itself as episodes of neurological dysfunction that remit spontaneously [relapsing–remitting MS (RRMS)] (1). Pathologically, relapses are associated with focal, inflammatory demyelination in white and gray matter, characterized by infiltration with macrophages and T and B lymphocytes (3). Over two-thirds of patients eventually develop secondary progressive MS (SPMS), a disease stage that is believed to be driven by neurodegeneration, where patients experience slow and irreversible accumulation of disability, predominantly affecting ambulation and cognition (1, 4). In a small percentage of MS patients, progression sets in at disease onset, a disease course that is termed primary progressive MS (1). The pathophysiology of primary and secondary progression remains largely unexplained; however, multiple lines of evidence suggest that progressive MS is associated with chronic activation of the CNS innate immune system (5–7). The poor understanding of the pathomechanisms underlying progression is reflected in the current treatment options for MS, with 13 FDA-approved medications being available for RRMS, one moderately effective medication for primary progressive MS, and none for secondary progression (8).

Multiple sclerosis is the result of an interplay between environmental and genetic factors. Genome-wide association studies (GWASs) have identified over 230 variants associated with susceptibility for MS that all confer small increases in disease risk (9–11). Environmental factors associated with MS risk include smoking, childhood obesity, low vitamin D levels, infection with the Epstein–Barr virus, and possibly a high salt diet (12–14). The pathological hallmark of MS is the presence of focal inflammatory lesions characterized by primary demyelination and relative preservation of axons (15). Acute demyelinating lesions are populated by abundant foamy, myelin-laden macrophages and by lymphocytes that are located in the perivascular space and diffusely throughout the lesion area, albeit at much lower numbers than myeloid cells (15). Acutely demyelinating lesions eventually evolve into chronic active lesions, which are characterized by completed demyelination and astroglial scarring in the lesion center, and inflammatory cells at

the lesion rim, possibly associated with low-grade demyelination (15). Finally, chronic silent lesions consist of astroglial scars with few or no inflammatory cells (15). Astrocytes have traditionally been assigned a bystander role, reacting only once demyelination is completed by forming a glial scar (16). However, recent literature suggests that astrocytes are early and highly active participants in MS lesion development (17–19). Astrocytes play multiple roles in the evolution of MS lesions, not only by recruiting lymphocytes (19, 20) and contributing to tissue damage (21–24) but also by confining inflammation and promoting lesion repair (18). In addition, astrocytes themselves sustain significant damage during the inflammatory process (16). This review focuses on the contributions of astrocytes to MS lesion formation. We discuss astrocytic phenotypes, prototypical pathways for astrocyte activation, including the impact of genetic risk variants for MS susceptibility on astrocyte responses, and mechanisms by which MS treatments affect astrocyte function.

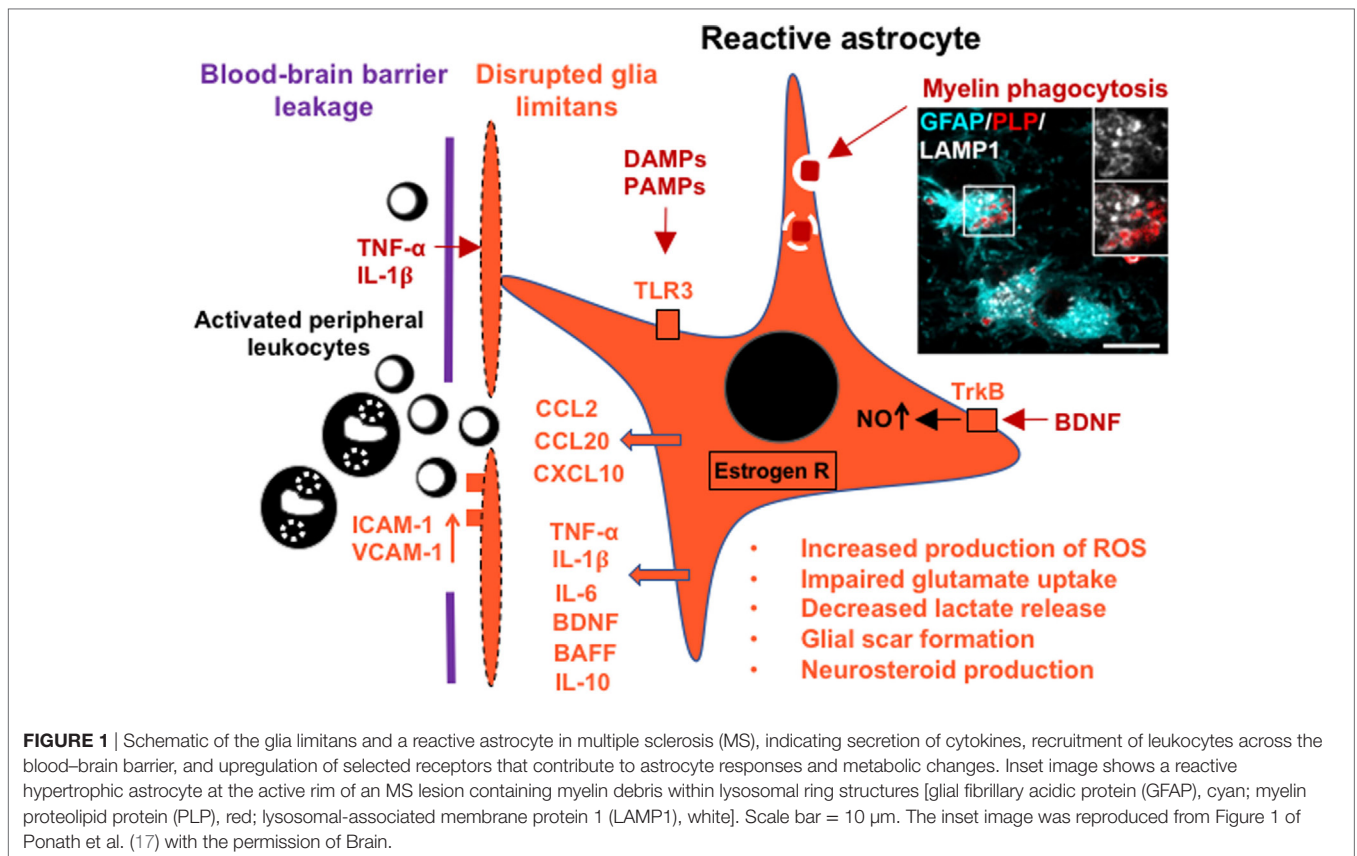## THE PHYSIOLOGICAL ROLE OF ASTROCYTES AND ASTROCYTE RESPONSES

Astrocytes make up approximately 30% of glial cells in the CNS, where each astrocyte occupies a unique territory demarcated by non-overlapping, star-shaped processes that extend from the cell soma (25, 26). The distal end feet of these processes form the glia limitans when they envelop the parenchymal basal lamina associated with blood vessels or meninges (18, 25). The glia limitans contributes to the maintenance of blood–brain barrier (BBB) integrity and forms a secondary barrier that further restricts entry of peripheral immune cells into the CNS (16, 27). Astrocytes are paramount for normal CNS functions, including maintenance of glutamate, extracellular potassium, and water homeostasis (20, 25). Astrocytes are functionally connected to adjacent astrocytes and to oligodendrocytes by gap junctions, thereby forming large syncytium-like glial networks that are composed of hundreds of cells (28). Together with neuronal synapses, astrocyte processes form so-called tripartite synapses, where one single astrocyte connects with tens of thousands of neuronal synapses (29) to regulate neuronal synaptic transmission, e.g., by releasing glutamate, D-serine, and ATP (30, 31). Astrocytes also prune synapses through phagocytosis (32) and modify gene expression, e.g., associated with neural plasticity, in surrounding neurons by secreting miRNA-containing exosomes (33). In addition, astrocytes secrete neurotrophic factors (34) and are metabolically coupled to neurons, releasing lactate for neuronal uptake and providing antioxidants such as glutathione and thioredoxin (35, 36). Astrocytes also participate in the production of neurosteroids, such as allopregnanolone, estrogen, and dehydroepiandrosterone (DHEA), that are synthesized in the nervous system, where they modulate neuronal excitability, promote myelination, and dampen pro-inflammatory responses in astrocytes (37–41). Moreover, in the healthy CNS, astrocytes contribute to an anti-inflammatory environment through constitutive low-level secretion of the anti-inflammatory cytokines TGF-β (42) and IL-10 (43), expression of Fas ligand (44, 45), and induction of upregulation of the co-inhibitory cell surface receptor CTLA-4 on helper T cells (46).

Astrocyte reactivity in adaptive and innate immune responses can be triggered through oxidative or chemical stress, pro-inflammatory cytokines, damage-associated molecular patterns (DAMPs), released in the context of CNS tissue damage, and pathogen-associated molecular patterns (PAMPs), such as double-stranded RNA and bacterial membranous endotoxins, released from pathogens (19, 47, 48). Stimulation of astrocytes induces or upregulates astrocytic secretion of cytokines, such as TNF-α, IL-1β, and IL-6; neurotrophic factors including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and leukemia inhibitory factor (LIF) (19, 48–50); chemokines including CCL2, CCL20, and CXCL10; and β-defensins, antimicrobial peptides that can directly diminish the stability of bacterial membranes and stimulate various immune functions (51, 52). In addition, reactive astrocytes express cell adhesion molecules such as ICAM-1 and VCAM-1 (50), inducible nitric oxide synthase (iNOS) with concomitant production of reactive nitrogen species (53, 54), and the PAMP-recognizing toll-like receptor 3 (TLR3), while other TLRs remain low to undetectable (55–58). Activation of TLR3 triggers a predominantly neuroprotective response, characterized by secretion of growth and differentiation mediators as well as pro- and anti-inflammatory cytokines (58).

Astrocytes also constitutively express low amounts of MHC-II and the adhesion molecules LFA-1 (CD11a) and ICAM-1 (CD54) (59). Stimulation with IFN-γ alone or in combination with TNF-α upregulates MHC-II, adhesion molecules, and co-stimulatory molecules B7-1 (CD80) and B7-2 (CD86) (60). Functional studies have shown that IFN-γ-treated murine astrocytes act as weak antigen-presenting cells, moderately activating CD4+ and CD8+ T cells. In contrast, cytokine-treated human astrocytes were not able to induce proliferation of encephalitogenic T cells, presumably because of lack of additional proliferation-inducing factors (59), suggesting interspecies differences in astrocytes. Furthermore, in the inflamed CNS, reactive astrocytes may contribute to B cell survival, maturation, and proliferation through production of B cell-activating factor of the TNF family (BAFF) (61–63). Other soluble factors secreted by astrocytes, such as IL-6 and IL-15, also support B cell survival (61). Finally, stimulation of astrocytes with cytokines diminishes their homeostatic and metabolic functions, resulting in impaired glutamate uptake, which may cause excitotoxicity, and in metabolic uncoupling from axons/neurons due to decreased release of lactate (23, 24, 64–66) (**Figure 1**).

Reactive astrocytes have recently been categorized according to their transcriptome profiles as "A1" or "A2," in analogy to the "M1" and "M2" phenotype categories for macrophages (67). A1-type astrocytes, for which complement component 3 is an identifying marker, are induced by inflammation (67, 68), are abundant in MS and neurodegenerative diseases, including Alzheimer's and Parkinson's disease, and secrete a neurotoxin that has not yet been identified (67). In contrast, A2-type

FIGURE 1 | Schematic of the glia limitans and a reactive astrocyte in multiple sclerosis (MS), indicating secretion of cytokines, recruitment of leukocytes across the blood–brain barrier, and upregulation of selected receptors that contribute to astrocyte responses and metabolic changes. Inset image shows a reactive hypertrophic astrocyte at the active rim of an MS lesion containing myelin debris within lysosomal ring structures [glial fibrillary acidic protein (GFAP), cyan; myelin proteolipid protein (PLP), red; lysosomal-associated membrane protein 1 (LAMP1), white]. Scale bar = 10 µm. The inset image was reproduced from Figure 1 of Ponath et al. (17) with the permission of Brain.

astrocytes are induced by ischemia and express neurotrophic factors (67). The concept of M1/M2 polarization is now considered an oversimplification and has been abandoned in favor of multiple, complex polarization states that do not necessarily align with a one-dimensional M1–M2 spectrum (69). Thus, while defining these phenotypes is an important step, reactive astrocytes may also exceed the A1–A2 dichotomy and assume a range of profiles with mixed A1 and A2 features (70). It has been proposed that although reactive astrocytes share common properties, they also display unique cellular and molecular features that are specific to different neuropathologies (70, 71). Moreover, distinct astrocytic phenotypes may coexist or develop sequentially during different phases of a pathological process: reactive astrocytes may first produce pro-inflammatory cytokines and reactive oxygen species in conjunction with hypertrophy and proliferation. In a second phase, astrocytes may promote anti-inflammatory and neuroregenerative functions through astrocyte-derived neurotrophic factors (72).

Thus, reactive astrocytes can mount powerful inflammatory responses that drive leukocyte recruitment to the CNS and thereby contribute to a successful defense against pathogens. Moreover, reactive astrocytes may change their response profiles over time, resulting in the secretion of anti-inflammatory cytokines and neurotrophins (72, 73). Astrocytosis can also aid in BBB repair and, depending on the severity of the injury, lead to the formation of glial scars that isolate the inflamed area, restrict damage and provide structural support (25).

THE REACTIVE ASTROCYTE IN MS LESION PATHOLOGY

Astrocytes are increasingly recognized as cells that critically contribute to the development of MS lesions. Previously, astrocytes were believed to react only at a late, post-inflammatory stage by forming a glial scar, but are now considered early and active players in lesion pathology (16, 17). In active lesions, astrocytes assume a hypertrophic morphology, characterized by massive enlargement of the cell soma and reduced process density (16). Typically, pronounced astroglial hypertrophy is indicative of substantial tissue injury and might be caused in MS lesions by oligodendrocyte loss and the resulting disruption of astrocyte–oligodendrocyte networks (16, 28). In addition, hypertrophic astrocytes may themselves sustain substantial damage that leads to retraction or loss of glia limitans from the basal lamina around blood vessels, presumably further increasing access of immune cells to the CNS (16) (**Figure 1**).

Reactive astrocytes are present in the active margins of demyelinating lesions and extend into adjacent, normal-appearing white matter (NAWM), suggesting that they are early contributors to lesion development (16, 17). This view is supported by the observation in murine experimental autoimmune encephalomyelitis (EAE), an inflammatory demyelinating model of MS, that astrocytes in nascent lesions become activated before significant immune cell infiltration into the parenchyma takes place (74–76). Furthermore, we have shown that hypertrophic astrocytes at

the leading edge of actively demyelinating MS lesions contain myelin debris (17). We have demonstrated that this myelin uptake induces astroglial NF-κB signaling and secretion of cell-recruiting chemokines. Therefore, we hypothesized that uptake of damaged myelin by astrocytes may be an early trigger for their activation, leading to astrocyte-mediated influx of leukocytes at the very beginning of lesion development (17) (**Figure 1**). Given that astrocytes in MS lesions express MHC class II and co-stimulatory molecules CD80 and CD86 (77, 78), it is tempting to speculate that myelin phagocytosis by astrocytes results in the presentation of myelin antigens to T cells. However, since stimulated human astrocytes in culture fail to induce, and even inhibit, proliferation of T cells (79), we consider it unlikely that astrocytes act as competent antigen-presenting cells in MS lesions.

In MS, hypertrophic astrocytes express chemokines and cell adhesion molecules associated with macrophage/microglia and lymphocyte recruitment into the parenchyma (80–82). Their functional relevance to leukocyte recruitment has been well documented in EAE. For example, in mice with a conditional, astrocyte-specific gene deletion of CCL2, induction of EAE resulted in a less severe disease course with fewer macrophage and T cell infiltrates, and less activation of astrocytes and microglia (83). Similarly, mice with a genetic deletion of all ICAM-1 isoforms showed marked attenuation of EAE, with minimal cellular infiltration and demyelination in the spinal cord (84). Conversely, astrocyte-mediated recruitment of microglia to demyelinating lesions is also of benefit, as demonstrated in a demyelination model using the oligodendrocyte toxin cuprizone, which does not disrupt the BBB or involve peripheral immune cell infiltration (85). Genetic ablation of astrocytes in mice treated with cuprizone prevented the recruitment of microglia cells to the site of demyelination, leading to delayed removal of myelin debris, impaired remyelination, and reduced proliferation of oligodendrocyte precursor cells (86). Thus, activated astrocytes are key regulators for the removal of damaged myelin, which is needed before remyelination can take place (86).

In addition, BAFF production by reactive astrocytes may contribute to the pathogenesis of MS by promoting B cell survival and proliferation in the CNS (61, 62). BAFF levels were shown to be increased in the CSF of MS patients compared to healthy controls (87). Moreover, BAFF mRNA was strongly upregulated in MS lesions, comparable to levels observed in lymphatic tissues, and BAFF was found to be expressed in reactive astrocytes, adjacent to inflammatory cells that expressed BAFF receptors (63). Given the continuous presence of antigen-experienced B cell clones in the CNS of MS patients (88) and the development of meningeal B cell follicles in progressive MS (89), astroglial production of BAFF may be a major factor to sustain these cells and to drive B cell-related pathology.

Reactive astrocytes likely contribute to tissue damage in MS through impaired glutamate handling and redox homeostasis. Glutamate concentrations were shown to be elevated in acute lesions of MS patients using *in vivo* MR spectroscopy (90). Moreover, a GWAS has linked specific risk variants associated with glutamate metabolism to increased cortical glutamate concentrations and poor disease outcomes in MS patients (91).

In EAE, disease severity as well as oligodendrocyte and neuronal death were ameliorated through treatment with antagonists to the AMPA/Kainate or NMDA type of glutamate receptors (23, 24).

A recent study in a chronic progressive model of EAE has shown that astrocytes produce and are stimulated by the sphingolipid lactosylceramide (LacCer) (7). LacCer induces production of pro-inflammatory cytokines and iNOS in astrocytes and promotes pathology during experimental spinal cord injury (92). In EAE, LacCer was found to control the recruitment and activation of microglia and CNS-infiltrating monocytes by astrocytes. In addition, inhibition of LacCer synthesis suppressed CNS innate immunity and neurodegeneration. Finally, LacCer and the LacCer synthase β-1,4-galactosyltransferase 6 (B4GALT6) were detected in reactive astrocytes within MS lesions (7), suggesting that the B4GALT6-LacCer pathway is relevant to human disease.

Although reactive astrocytes drive inflammatory and neurotoxic responses in MS lesions, they may also dampen inflammation and promote neuroprotection and lesion repair. A factor produced by astrocytes and neurons in the normal CNS, which has CNS-trophic effects, is BDNF (93, 94). In EAE, astrocyte-specific deletion of BDNF resulted in a more severe clinical course with increased axonal loss (95). Moreover, in the cuprizone mouse model, enhanced BDNF production by astrocytes, induced by stimulation of metabotropic glutamate receptors, resulted in enhanced remyelination (96). However, a separate study demonstrated that signaling through the BDNF receptor TrkB in astrocytes leads to production of nitric oxide (NO) (97). EAE induced in mice with astrocyte-specific genetic deletion of TrkB had ameliorated disease severity, concomitant with reduced expression of astrocytic and lesional iNOS (97). These data indicate that BDNF released by astrocytes not only elicits neuroprotective effects in other cell types but also stimulates production and release of toxic NO in astrocytes themselves. In MS lesions, BDNF is primarily present in immune cells and reactive astrocytes (98), while the BDNF receptor TrkB was strongly upregulated in reactive astrocytes and in neurons in the immediate lesion vicinity (98). This suggests a possible dual protective and degenerative role for BDNF.

Astrocytes are susceptible to neurosteroids, such as estrogen and DHEA, which downregulate pro-inflammatory responses in reactive astrocytes (99–101). This mechanism plays a significant role in EAE where treatment of mice with an estrogen receptor-α (ERα) ligand substantially ameliorated clinical symptoms, inflammatory infiltrates, and axonal loss (102, 103). These beneficial effects were mediated entirely through ERα expressed by astrocytes, as they were abolished in EAE induced in mice with conditional, astrocyte-specific deletion of ERα (103). In MS lesions, ERα, aromatase, an enzyme involved in estrogen synthesis, and progesterone receptor were found to be upregulated in reactive astrocytes (104), suggesting that neurosteroid synthesis by reactive astrocytes as well as astrocytic responses to neurosteroids are part of an endogenous protective mechanism. On the other hand, a recent study found that the neurosteroids allopregnanolone and DHEA were substantially downregulated in EAE and in NAWM of autopsied MS tissue (105). Provided that astrocytes are the main steroidogenic cells in the brain (38), these

data may point toward impaired synthesis of both neurosteroids by astrocytes in MS.

In addition, TLR signaling may play a neuroprotective role in EAE and by extension, in MS, although this effect might not be astrocyte-specific. Systemic administration of the TLR3 agonist polyinosinic:polycytidylic acid (poly I:C) in EAE suppresses relapsing demyelination through induction of IFN-β and other immune regulatory effects (106). Furthermore, TLR4 knockout mice exhibited more severe EAE symptoms than wild-type mice, associated with increased priming of encephalitogenic Th17 cells (107). In MS lesions, TLR3 and 4 are expressed by microglia and astrocytes, where astroglial TLR expression is particularly prominent at later stages of inflammation, which may be instrumental in mitigating inflammation and promote tissue repair (56, 58).

Furthermore, following acute inflammation and demyelination, hypertrophic astrocytes eventually form a glial scar in the center of white matter lesions (25). While scars have been considered as barriers to tissue regeneration (16), they also provide beneficial features and contribute to recovery from CNS insults (25). For example, glial scars support demyelinated axons, help restore BBB function, and confine inflamed areas, preventing the spread of immune cells and toxic levels of extracellular ions, metabolites, or DAMPs into healthy tissues or areas of repair (16, 25).

Recent studies have implicated gut microbiota in immunological disorders including MS and its animal model, EAE (108, 109). The microbiome has emerged as a regulator of BBB integrity, where the absence of normal gut flora leads to disorganization of tight junctions in endothelial cells (110), and the production of short-chain fatty acids by bacteria corrects BBB dysfunction (111, 112). However, to date, astrocytes have not been found to mediate these effects.
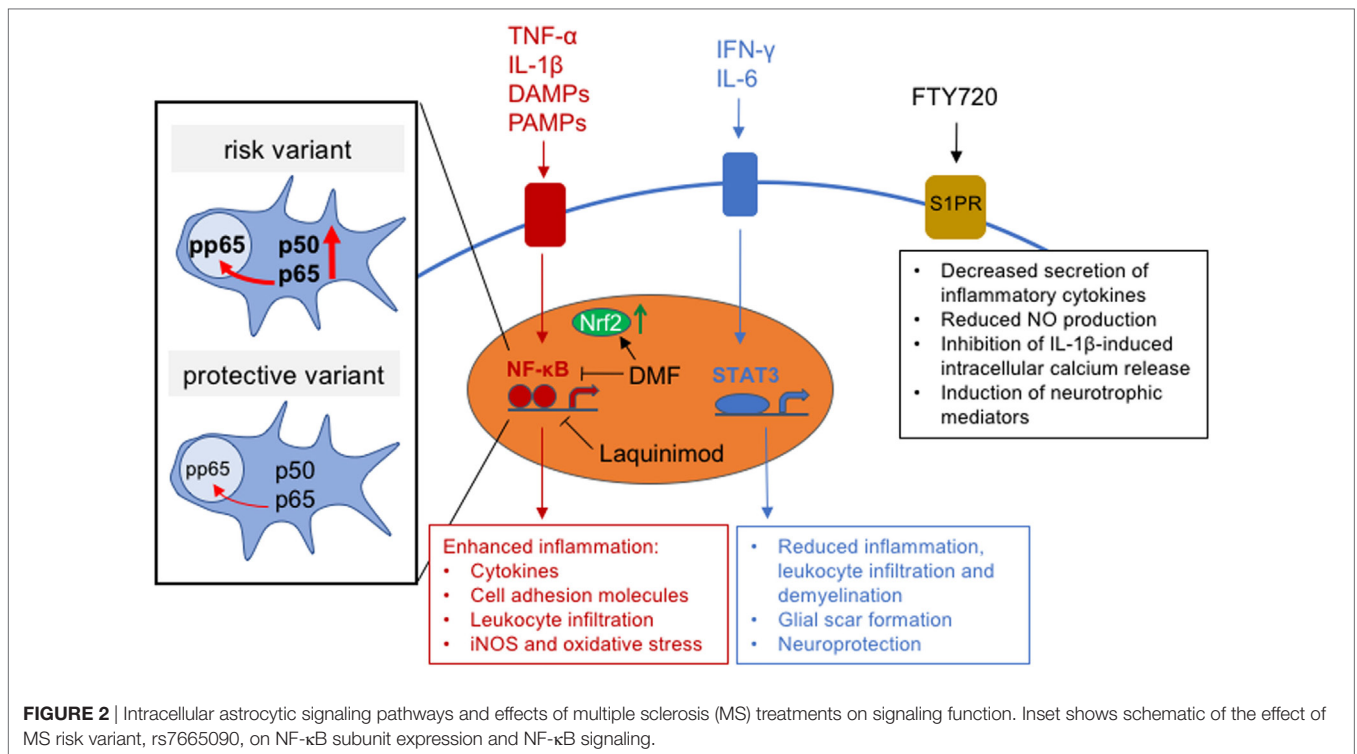
SIGNALING PATHWAYS IN ASTROCYTES

Astrocyte reactivity is regulated by key canonical signaling cascades, among which the NF-κB pathway is pivotal for establishing neuroinflammation (113) (**Figure 2**). NF-κB is a master regulator of innate and adaptive immunity that controls cell survival, differentiation, and proliferation (114). Astrocytic NF-κB signaling is directly activated through stimulation with the pro-inflammatory cytokines TNF-α and IL-1β (113), through TLR signaling and various other agents including phagocytosed myelin, mitogens, and free radicals (17, 113, 115, 116). NF-κB signaling in astrocytes plays a critical role for initiating and maintaining inflammation in the CNS. Transgenic mice with astrocyte-specific inactivation of NF-κB display dramatic amelioration of tissue damage and clinical impairment following induction of EAE, spinal cord injury, or ischemic retinal injury compared to wild-type mice (117–119). Similarly, ablation of IL-17-induced Act1 signaling in astrocytes, which abolishes IL-17-mediated NF-κB activation, reduces the recruitment of lymphocytes and macrophages and markedly ameliorates disease severity in EAE (120).

Interestingly, microbial flora and its products have been shown to control NF-κB signaling through conversion of dietary tryptophan into agonists of aryl hydrocarbon receptor (AHR), which is highly expressed by astrocytes (121). AHR suppresses

the classical activation pathways of NF-κB through competitive binding to the NF-κB subunit p65 (122). Induction of EAE in mice with astrocyte-specific genetic knockout of AHR [glial fibrillary acidic protein (GFAP)–AHR⁻] resulted in increased expression of chemokines, cytokines and pro-inflammatory markers and an exacerbated disease course compared to wild type animals. Moreover, mice fed with a tryptophan-depleted diet exhibited a more severe EAE course, which could not be reversed by addition of tryptophan in GFAP–AHR⁻ mice. In MS, *AHR* expression was upregulated in active and chronic MS lesions and localized to GFAP⁺ astrocytes; however, this might not translate into AHR-dependent downregulation of astrocyte activation, because expression of the AHR transcriptional target *CYP1B1* was decreased in MS lesions and NAWM, suggesting that this pathway is defective in MS (121).

We have recently shown that an MS risk variant, rs7665090, which increases NF-κB signaling in lymphocytes (123), substantially affects astrocyte reactivity in cell culture and MS white matter lesions (81). Astrocytes derived from induced pluripotent stem cells, obtained from MS patients carrying the risk variant, showed increased NF-κB activation, chemokine and cell adhesion molecule expression, as well as impaired glutamate uptake and reduced lactate release. In addition, the risk variant was associated with significantly higher numbers of infiltrating lymphocytes in white matter MS lesions and with an increased lesion load on MRI in MS patients (81). Therefore, this NF-κB-relevant risk variant promotes pro-inflammatory changes in astrocytes that might help target aberrant immune responses to the CNS. This challenges the view that MS is mediated solely through dysregulation of lymphocytes and highlights the importance of astroglial NF-κB signaling for lesion formation (81) (**Figure 2**).

An important but less elucidated signaling cascade for regulation of astrocyte activation in MS is the STAT3 pathway (**Figure 2**). STAT3 activity is generally upregulated in response to CNS inflammation and damage (124). In astrocytes, STAT3 signaling is induced by both pro- and anti-inflammatory molecules, including IFN-γ and cytokines of the IL-6 family, that bind to the gp130 cell-surface receptor to induce STAT3 phosphorylation (124–127). STAT3 signaling in astrocytes plays a beneficial role in CNS inflammation, as demonstrated in mice with conditional, astrocyte-specific knockout of STAT3, where spinal cord injury lesions exhibited increased demyelination, contained more infiltrating dendritic cells, and had attenuated astrocyte hypertrophy and glial scar formation (128, 129). Similarly, in EAE, disease severity was exacerbated in mice with astrocyte-specific knockout of the STAT3-activating gp130 signal transducer, with larger areas of demyelination and increased infiltration of reactive T-lymphocytes (130). Moreover, activated astrocytes have been shown to provide neuronal protection *via* ERK (131) and/or STAT3 signaling during inflammation (132). This was demonstrated in an *in vivo* model of acute LPS/IFN-γ-induced neuroinflammation, where STAT3 and ERK signaling induced IL-6 production, which protected against neuronal apoptosis (133). Despite its importance as a neurotrophin in the CNS, IL-6 is also known to promote MS lesion development when produced in excess (134, 135). Specifically, IL-6 inhibits differentiation of naïve T cells into regulatory T cells and promotes their differentiation into

FIGURE 2 | Intracellular astrocytic signaling pathways and effects of multiple sclerosis (MS) treatments on signaling function. Inset shows schematic of the effect of MS risk variant, rs7665090, on NF-κB subunit expression and NF-κB signaling.

Th17 helper cells, which are considered to be major mediators of MS pathology (134). Thus, IL-6 levels above or below a certain threshold may determine its role as either a growth factor and activator of STAT3-mediated anti-inflammatory function, or as a suppressor of regulatory T cell differentiation and enhancer of pro-inflammatory helper T cell activity. Therefore, understanding the dynamics of IL-6 production in CNS lesions may help to predict the effectiveness of STAT3 signaling as a suppressor of lesion pathology.

DIRECT MODULATION OF REACTIVE ASTROCYTE ACTIVITY BY MS TREATMENTS

As discussed above, activated astrocytes play multiple pivotal roles during inflammation, including regulation of leukocyte trafficking, release of neurotoxic factors, confinement of inflammation, and promotion of neuroprotection and tissue repair. This makes astrocytes obvious therapeutic targets in MS. Ideally, such treatments would take into account the multi-functionality of astrocytes to block detrimental responses and/or enhance regenerative properties. Current MS therapies that are known to cross the BBB and modulate astrocyte function are laquinimod, which is currently being developed as an MS treatment, dimethyl fumarate (DMF; Tecfidera®) and fingolimod (FTY720; Gilenya®)/siponimod. In clinical studies, siponimod and laquinimod have shown a positive impact on progressive MS and brain atrophy, respectively. Since progressive MS is believed to be driven in part

by chronic glial activation, these studies provide circumstantial evidence that astrocyte activation may contribute to progressive pathology. Below, we provide details of how each of these compounds impacts astrocytes.

Laquinimod

Laquinimod is a small quinolone derivative of the immunomodulatory compound linomide. Laquinimod was initially tested in RRMS, where it led to moderate effects on the reduction of relapse rates as a primary study endpoint. However, significant effects were observed on brain atrophy and disease progression (136). This led to a clinical trial of laquinimod in primary progressive MS (ARPEGGIO trial), which is still ongoing (137).

While the precise molecular targets of laquinimod are not well defined, recent data suggests that laquinimod activates genes associated with the transcription factor aryl hydrocarbon receptor (AHR, see above) (138). AHR target genes encode for drug-metabolizing enzymes and proteins controlling cell proliferation, differentiation, and apoptosis (139, 140). Additionally, cross talk between AHR and other signaling pathways, including epidermal growth factor receptor (EGFR) (141, 142), protein kinase A (PKA) (143, 144), and NF-κB signaling (144, 145), has been reported (146). Accordingly, AHR modulates the differentiation and function of many cell populations, several of which play an important role in neuroinflammation. In mouse EAE, laquinimod exerts effects on the peripheral immune system, where it downregulates pro-inflammatory T cell responses (147, 148), and on CNS cells. Genetic deletion of AHR in the immune system fully abrogated the treatment effect of laquinimod on

EAE, while deletion of AHR in the CNS partially abrogated this effect (138). In a separate study, laquinimod markedly reduced NF-κB signaling and pro-inflammatory activation of astrocytes, but not of microglia *in vitro* (**Figure 2**). In the cuprizone model of demyelination, laquinimod prevented demyelination, microglial activation, T cell infiltration, and axonal transection; this effect was attributed to *in vivo* attenuation of NF-κB signaling in astrocytes (149). Laquinimod exhibits additional modes of action including neuroprotection, as demonstrated in EAE, where conditional deletion of BDNF in myeloid and T cells partially abrogated the beneficial effect of laquinimod. Similarly, laquinimod treatment of MS patients was found to increase expression of BDNF in serum (150). Taken together, current data suggests that laquinimod exerts effects on multiple cell types during CNS inflammation. A key mechanism mediated by laquinimod is the downregulation of pro-inflammatory responses in astrocytes. Thus, the beneficial effects of laquinimod on brain atrophy and disability progression in MS patients may at least partially be explained by its direct effect on astrocytes.

Dimethyl Fumarate

Dimethyl fumarate is the methyl ester of fumaric acid and was FDA-approved for the treatment of relapsing forms of MS in 2013. In placebo-controlled clinical trials, Tecfidera® reduced the relapse rate in MS patients by approximately 50% and disability progression by 38% in one trial but not in a separate, parallel trial (151, 152). The effect of Tecfidera® on SPMS is unclear, as a phase III clinical trial was initiated but terminated early due to restructuring of the drug's manufacturer, Biogen (153). DMF activates the Nrf2 transcription factor, which targets antioxidant response element (ARE) genes coding for antioxidant enzymes that reduce oxidative stress (154). DMF induces Nrf2 through glutathione depletion and direct binding to the Nrf2 repressor Kelch-like ECH-associated protein 1 (KEAP1) (155–158). Moreover, DMF acts as a potent inhibitor of NF-κB signaling (159) and has been shown to modify DMF-sensitive cysteine residues in human T cells, which inhibits T cell activation (160) (**Figure 2**).

In the peripheral immune system, DMF reduces lymphocyte counts, in particular cytotoxic and effector T cells, and inhibits activation of antigen-presenting cells (161). In the CNS, a major effect of DMF is the upregulation of Nrf2 in astrocytes, which is protective against oxidative injury *via* upregulation of oxidative stress-induced growth inhibitor 1 (162). This effect might ameliorate astrocytic damage in active lesions, including the retraction of perivascular astrocyte end feet along basal lamina (16), to reduce leakage across the BBB and the cortical surface (163). DMF also inhibits secretion of pro-inflammatory cytokines and chemokines by astrocytes and microglial cells, independent of changes in antioxidant gene expression (164). Therefore, in addition to its effect on the peripheral immune system, DMF has a direct impact on the CNS that involves protective and anti-inflammatory effects on astrocytes.

Fingolimod and Siponimod

FTY720/fingolimod(2-amino-2[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride; Gilenya®) is a non-specific sphingosine-I

phosphate (S1P) modulator. In clinical trials with RRMS patients, FTY720 reduced the annualized relapse rate by 48–55% and decreased risk of disability progression by 28% in one study, while having no significant effect on disability in another (165, 166). With regards to primary progressive MS, a recent trial demonstrated that FTY720 had no beneficial effects on disability progression or whole-brain atrophy (167). However, Siponimod, a selective modulator of the $S1P_1$ and $S1P_5$ receptors, did slow disability progression in SPMS in a phase III clinical trial (168).

The main effect of FTY720 on the peripheral immune system is the internalization and degradation of the S1P receptor on lymphocytes, which results in impaired responses to the S1P gradient in lymph nodes and prevents lymphocyte egress (169, 170). In the CNS, S1P receptors play a number of roles in brain cell function, including astrocyte proliferation and migration (171, 172), oligodendrocyte differentiation and survival (173, 174), and neurite outgrowth and neurogenesis (175–177). The mechanism most relevant to MS and its animal model, EAE, involves $S1P_1$ receptor signaling in astrocytes, which has been demonstrated in conditional null mouse mutants lacking $S1P_1$ in astrocytes. When induced to develop EAE, these mice showed a substantial reduction in disease severity, which was not further affected through additional FTY720 treatment, suggesting that the main effect of FTY720 in EAE involves modulation of astrocyte function but not the arrest of lymphocytes in lymph nodes (178). In contrast, conditional deletion of $S1P_1$ in neuronal cell lineages had no impact on EAE severity or the efficacy of FTY720 to suppress EAE. Astrocytes mainly express $S1P_1$ and $S1P_3$ as well as other subtypes at low levels (171, 179). Expression of both receptors is markedly increased in reactive astrocytes in active and chronic MS lesions. Moreover, treatment of cultured human astrocytes with FTY720 limits secretion of inflammatory cytokines (180), reduces NO production (181), induces neurotrophic mediators, and inhibits TNF-α-induced inflammatory gene expression (182). Modulation of astrocytic intracellular pathway function induced by FTY720 includes enhanced expression of calcium-regulating proteins and inhibition of calcium release induced by the pro-inflammatory mediator IL-1β (183) (**Figure 2**). This data implicates $S1P_1$ signaling in astrocytes as a major contributor to the pathogenesis of EAE and as the main therapeutic target of FTY720 (184). Thus, the efficacy of Siponimod, a modulator of the $S1P_1$ and $S1P_5$ receptors, in slowing disability accumulation in SPMS may be mediated through its direct effect on astrocytes.

Other MS therapies, such as teriflunomide (Aubagio®) (185, 186) and IFN-β (Avonex®, Betaseron®, Rebif®), have been shown to inhibit astroglial immune responses, the latter by inducing astroglial expression of AHR (121). However, teriflunomide and IFN-β have limited or no BBB penetrance (187, 188), making it unlikely that these drugs exert continuous, direct effects on CNS-resident cells.

CONCLUSION

Astrocytes play an instrumental role in the formation of MS lesions through a multitude of functional changes associated

with their activation. Astrocytes are early responders in nascent white matter lesions, are the main recruiters of lymphocytes, and act themselves as immunocompetent cells that contribute to innate immunity. Moreover, astrocytes not only can adopt a neurotoxic phenotype, but also confine inflammation through scar formation and can promote neuroprotection and tissue repair. Astrocytic dysfunction associated with a genetic MS risk variant further suggests that astrocyte-mediated processes are causative in lesion pathology. Thus, while MS is driven by dysfunction of the peripheral immune system, CNS cells such as astrocytes may contribute to MS pathology by targeting dysregulated immune responses to the CNS. Finally, MS medications that impact astrocytes have shown efficacy in both relapsing–remitting and phase III clinical trials of progressive MS, providing further circumstantial evidence that activation of astrocytes contributes to both pathologies.

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GP, CP and DP wrote the manuscript.

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