Acta Neuropathologica (2019) 137:757–783
https://doi.org/10.1007/s00401-019-01980-7

REVIEW

The contribution of astrocytes to the neuroinflammatory response in multiple sclerosis and experimental autoimmune encephalomyelitis

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Received: 4 February 2019 / Revised: 21 February 2019 / Accepted: 23 February 2019 / Published online: 7 March 2019

Abstract

Neuroinflammation is the coordinated response of the central nervous system (CNS) to threats to its integrity posed by a variety of conditions, including autoimmunity, pathogens and trauma. Activated astrocytes, in concert with other cellular elements of the CNS and immune system, are important players in the modulation of the neuroinflammatory response. During neurological disease, they produce and respond to cellular signals that often lead to dichotomous processes, which can promote further damage or contribute to repair. This occurs also in multiple sclerosis (MS), where astrocytes are now recognized as key components of its immunopathology. Evidence supporting this role has emerged not only from studies in MS patients, but also from animal models, among which the experimental autoimmune encephalomyelitis (EAE) model has proved especially instrumental. Based on this premise, the purpose of the present review is to summarize the current knowledge of astrocyte behavior in MS and EAE. Following a brief description of the pathological characteristics of the two diseases and the main functional roles of astrocytes in CNS physiology, we will delve into the specific responses of this cell population, analyzing MS and EAE in parallel. We will define the temporal and anatomical profile of astroglial activation, then focus on key processes they participate in. These include: (1) production and response to soluble mediators (e.g., cytokines and chemokines), (2) regulation of oxidative stress, and (3) maintenance of BBB integrity and function. Finally, we will review the state of the art on the available methods to measure astroglial activation in vivo in MS patients, and how this could be exploited to optimize diagnosis, prognosis and treatment decisions. Ultimately, we believe that integrating the knowledge obtained from studies in MS and EAE may help not only better understand the pathophysiology of MS, but also uncover new signals to be targeted for therapeutic intervention.

Keywords Neuroinflammation · Neuroimmune disease · Astroglia · Multiple sclerosis · Experimental autoimmune encephalomyelitis · Demyelinating disorder

Abbreviations

AhR	Aryl hydrocarbon receptor
APC	Antigen presenting cell
AQP4	Aquaporin-4
BBB	Blood–brain barrier
BDNF	Brain-derived neurotrophic factor
CFA	Complete Freund's adjuvant
CIS	Clinically isolated syndrome
CLD5	Claudin-5
CNS	Central nervous system
CSF	Cerebro-spinal fluid
Cx43	Connexin 43
EAE	Experimental autoimmune encephalomyelitis
ERα	Estrogen receptor α
GFAP	Glial fibrillary acidic protein
GM	Gray matter
HB-EGF	Heparin-binding epidermal growth factor
ICAM-1	Intercellular adhesion molecule-1
IFN-I	Type I interferons
Ins	*Myo*-Inositol
LacCer	Lactosylceramide
MOG	Myelin oligodendrocyte glycoporotein

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MBP	Myelin basic protein
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MS	Multiple sclerosis
NADPH	Nicotinamide adenine dinucleotide phosphate
NAGM	Normal appearing gray matter
NAWM	Normal appearing white matter
NGF	Nerve growth factor
NO	Nitric oxide
NOS2	Nitric oxide synthase 2
OCLN	Occludin
OPC	Oligodendrocyte precursor cell
PET	Positron emission tomography
PLP	Proteolipid protein
PPMS	Primary progressive multiple sclerosis
ROS	Reactive oxygen species
RRMS	Relapsing–remitting multiple sclerosis
S1P	Sphingosine 1-phosphate
SPMS	Secondary progressive multiple sclerosis
tCr	Creatine
TGFβ	Transforming growth factor β
tNAA	*N*-acetylaspartate
TNF	Tumor necrosis factor
memTNF	Membrane-bound tumor necrosis factor
solTNF	Soluble tumor necrosis factor
WM	White matter
VEGF-A	Vascular endothelial growth factor A

Introduction

Astrocytes are key players in the complex cascade of cellular adaptations taking place in the central nervous system (CNS) in response to injury and disease. These adaptations, often referred to globally as neuroinflammation, occur in multiple sclerosis (MS) as well as its commonly used animal model experimental autoimmune encephalomyelitis (EAE), which has been successfully employed to investigate this and other aspects of MS immunopathology. Neuroinflammation is the coordinated response of the CNS to threats to its integrity posed by a variety of conditions, including pathogens and trauma. Activated astrocytes act in concert with other neural and non-neural cells to sustain the neuroinflammatory response by balancing cellular signals that often lead to opposing processes. As a result, the neurological outcome is dictated by the net combination of all effects, which tips the scale towards propagation or resolution of the damage at a specific time and place.

In recent years, many advances have been made in our understanding of astroglial biology, spearheaded by the development of new cutting edge tools, including gene-targeting approaches and next-generation transcriptomics. We now have an appreciation for the vast heterogeneity of the astroglial populations throughout the CNS and the timing and nature of their responses during disease. Because astrocyte-driven neuroinflammation is such a key feature of both MS and EAE, here we will review the astrocyte-specific responses observed in both, focusing on how they contribute to the neuroinflammatory cascades at the basis of disease onset, evolution and resolution.

Multiple sclerosis immunopathology

MS is a chronic inflammatory demyelinating disease of the CNS, whose underlying cause remains uncertain [129]. It is the most common non-traumatic neurological disorder in young adults, affecting an estimated 1 million people in the US alone [192]. MS is believed to be initiated and sustained by the complex interplay of dysregulated innate and/or adaptive immunity, genetic susceptibility and environmental factors. MS manifests with distinct clinical phenotypes, the most frequent being relapsing–remitting MS (RRMS), characterized by episodes of neurological dysfunction that spontaneously resolve [57]. Pathologically, relapses are associated with focal, inflammatory demyelination in white and gray matter, heavily infiltrated with immune cells. In over 75% of cases, RRMS evolves into secondary progressive MS (SPMS), where patients experience irreversible accumulation of disability associated with neurodegeneration [129]. In a small percentage of cases, a primary progressive phenotype is observed (PPMS), where irreversible and progressive neurodegeneration starts at onset. In progressive MS forms, chronic demyelinated lesions with axonal loss accumulate in the white matter, but diffuse changes also occur in the seemingly unaffected white matter, commonly referred to as normal-appearing white matter. Diffuse and focal cortical gray matter demyelination and neurodegeneration are also seen [89, 150]. While major progress has been made in understanding disease mechanisms in RRMS, those driving progressive MS remain largely unresolved, which explains the lack of effective treatments for this disease forms. In this respect, a breakthrough came with the recent introduction of the B cell depleting biologic ocrelizumab, which became the first, and so far only, drug approved for PPMS, underscoring the importance of B cell function in MS pathogenesis [63].

In addition to the key inflammatory role of the different T and B cell subpopulations and other elements of the peripheral innate immune system (e.g., monocytes, dendritic cells), CNS glial cells, namely astrocytes and microglia, have been recognized as key components of MS immunopathology. This seems especially evident in progressive MS, where multiple lines of evidence suggest an association with chronic activation of the CNS innate immune system [91]. Furthermore, several studies point at the possibility that, at least in certain cases, MS may initially arise from a primary insult within the CNS, perhaps to oligodendrocytes. This

may lead to glial activation and eventually immune-mediated inflammatory activation as a secondary phenomenon [105].

Experimental autoimmune encephalomyelitis: induction and pathological features

In its various forms, EAE has been the most widely utilized model of MS for decades [47]. It can be induced in multiple animal species, though rodents, particularly mice and rats, are usually preferred. This is due to their versatility, relatively low maintenance cost and potential for genetic manipulation that allows for investigations into specific cellular and molecular mechanisms of disease. Species, genetic strain, sex and age are factors that dictate the EAE phenotype that will manifest, whether with a chronic monophasic or a relapsing–remitting profile. EAE induction can be active or passive. Active EAE is obtained via direct immunization with CNS-specific antigens, namely highly immunogenic myelin peptides (e.g., $MOG_{35–55}$, $MBP_{84–104}$, $PLP_{139–151}$) emulsified in complete Freund's adjuvant (CFA) and usually accompanied by administration of pertussis toxin [47]. This leads to the priming of myelin-specific T cells in peripheral lymphoid organs, their differentiation into effector T cells (mostly Th1 and Th17) and entry into the CNS where they are reactivated via interaction with local antigen presenting cells (APCs). These events are followed by secretion of pro-inflammatory mediators from T cells, microglia and astrocytes, which further amplify the inflammatory response by sustaining the recruitment of more immune cells into the CNS [20]. Here, they accumulate predominantly in the white matter (especially in the spinal cord) where they cause demyelination and tissue damage. Passive EAE requires adoptive transfer of antigen-specific T cells obtained from actively immunized animals into recipient animals [47, 135]. Passive EAE is valuable to study the contribution of $CD4^+$ T cells in the immunopathology of EAE/MS and to investigate the effector phase of disease in the absence of adjuvant. Amongst the various EAE models, active EAE induced with $MOG_{35–55}$ peptide in C57BL/6 mice is by far the most common. Here, a monophasic response is observed, whereby, after a peak disease reached acutely, the clinical signs ameliorate to some degree to be then sustained chronically. Importantly, MOG immunization elicits not only an encephalitogenic T cell response, but also a demyelinating autoantibody response which enhances the severity of demyelination [95]. Anti-MOG autoantibodies are also found in MS patients and have been shown to induce demyelination when inoculated into rodents [203].

Both active and passive EAE models are not ideal to study immunological mechanisms at the basis of disease initiation and relapse. To overcome their limitations, transgenic models in which mice develop a spontaneous form of EAE have been generated. The first of these models was obtained via expression of a MBP-specific T cell receptor (TCR), where mice develop spontaneous EAE at various ages in about 50% of cases [62]. A number of similar models have since followed, in which other myelin-specific T cell receptors are expressed. These mouse lines can be crossed with RAG-deficient mice to obtain modified TCR expression in T cells in the absence of any other lymphocyte population [20]. Each line develops spontaneous EAE differently, and may require a triggering agent [20].

Regardless of the type of EAE, its pathological features include perivascular accumulation of immune cells in the CNS (especially at early disease stage) followed by their egress into the CNS parenchyma (maximal at disease peak) [29, 84], activation of microglia and astrocytes (throughout disease), demyelination (starting at peak disease, most evident chronically) and axonal loss (late chronic stage) [58]. The degree of immune cell entry and tissue damage directly correlates with functional impairment. Even though these immunopathological features are common to all EAE phenotypes, their timing, severity and distribution may vary significantly depending on the species and strain used, as well as the immunizing agent. For this reason, each individual EAE model is suited for investigating specific aspects of the human pathology. Overall, since EAE is primarily T cell driven and adaptive immune activation plays an integral role in its etiology, it is more amenable to study the induction and acute phases of RRMS, where accumulation of immune cells in active CNS lesions is the key driver of pathology and disability. The progressive MS forms where irreversible axonal injury and neurological damage are the predominant features, often observed in the absence of immune cell presence, are not modeled as accurately by EAE.

Similarly to MS, EAE features profound inflammatory activation of CNS glial cells, which largely contribute to EAE pathology at all stages of the disease. For this reason, EAE has been especially valuable in dissecting the processes and mechanisms driven by astrocytes, as well as other glia, in neuroimmune disease, thus helping to provide a more complete picture of MS pathophysiology.

Astrocyte function in health and disease

Over the last few decades, considerable efforts have been made to elucidate the complex functions of astrocytes in the healthy and diseased CNS. It is now established that astrocytes play essential roles that go far beyond the simplistic view of "supporting elements" to neurons. In fact, astrocytes are recognized to participate in functions once deemed to be exclusive prerogative of neurons, such as synaptic transmission and processing [171]. Astrocytes are found in a multitude of phenotypes (e.g., protoplasmic, fibrous, radial, velate, Bergmann glia, Muller glia) with diverse morphology, anatomical location and properties. They localize in all

CNS districts, occupying discrete non-overlapping domains where they carry out homeostatic functions during development and adulthood [33, 187]. Their highly ramified processes make contacts with pre- and post-synaptic terminals forming the tripartite synapse [12]. Within this structure, astrocyte regulate synaptogenesis [43], synaptic plasticity and stability, and the efficiency of synaptic transmission by modulating the release of gliotransmitters (e.g., glutamate, ATP, D-serine) in the synaptic cleft [11], uptaking glutamate [132], and buffering extracellular K^+ [70]. By extending and wrapping their end-feet around the cerebral vasculature, astrocytes constitute a key structural component of the blood–brain barrier (BBB). Indeed, they participate in the formation of the glia limiting membrane, or glia limitans, a dense meshwork of processes covered by basal lamina that makes contact with the pia mater and regulates the movement of small molecules and cells into the CNS [169]. Key molecules expressed by the astrocytic end-feet at the glia limitans are the specialized channel-forming proteins aquaporin-4 (AQP4) and Kir4.1, and the gap junction-forming protein Connexin 43 (Cx43). They allow astrocytes a direct exchange with endothelial cells, conferring them the ability to regulate water, ion and soluble factor diffusion and redistribution across the BBB [81]. Via local production of vasoactive molecules [e.g., prostaglandins, arachidonic acid, nitric oxide (NO)], astrocytes regulate CNS blood flow [85]. They manage extracellular fluid composition and pH through the activity of specialized water and ion channels [165]. As they are positioned at the interface between blood vessels and neurons, astrocytes can uptake glucose from the circulation and provide it to neurons for energy supply [187]. Furthermore, they are the main storage sites of glycogen in the CNS and the highest accumulation of astrocytic glycogen occurs in areas of high synaptic density [142]. Lipid metabolism is also regulated by astrocytes, who are the primary source of CNS cholesterol needed for membrane homeostasis and myelin synthesis [35, 36]. Finally, astrocytes are recognized as important components of the innate immune response. Indeed, they participate in immunomodulatory functions through their ability to produce cytokines and chemokines, as well as to express MHC-II molecules. They have been attributed a role as APCs, although it is now recognized they act as weak APCs in vivo given their lack of expression of certain co-stimulatory molecules [40, 46].

Astrocytes respond to CNS disease and trauma with a complex process of activation that integrates cell proliferation, profound morphological changes (e.g., increased branching, augmented cellular size, elongation of cells processes) and functional modifications. This phenomenon, often defined as astrogliosis, bares both positive and negative repercussions on the neurological outcome [139]. A commonly used marker to identify astrogliosis is the intermediate filament glial fibrillary acidic protein (GFAP), whose upregulation can be reliably correlated with the acquisition of a reactive/astrogliotic phenotype by astrocytes during CNS stress and disease. Recently, it has been suggested that reactive astrocytes display two distinct phenotypes, A1 and A2. While A2 astrocytes have been attributed neuroprotective and anti-inflammatory properties, A1 astrocytes induced by neuroinflammatory microglia during CNS disease are highly neurotoxic [93].

Because of the vast diversity of astrocyte populations in the various areas of the brain and spinal cord, it is now recognized that the astroglial response to CNS damage is uniquely region- and disease-specific. For instance, in CNS autoimmunity, the processes astrocytes participate in are initiated by the multitude of molecules released by CNS damaged cells, neighboring glia and infiltrating immune cells, especially T cells. These affect the makeup of astrocytes resulting in gain or loss of functional properties. These include the production of soluble mediators such as chemokines (e.g., CCL2, CCL20, CXCL1), cytokines (e.g., TNF, IL1β, IL6), growth factors (e.g., NGF, BDNF, VEGF-A), and oxidants (NO), as well as the synthesis of insoluble matrix molecules (e.g., tenascin, chondroitin–sulphate proteoglycans), whose deposition in the extracellular space leads to the formation of the glial scar [170]. As the processes activated in reactive astrocytes during CNS autoimmunity are both detrimental and reparative, the roles these cells play in disease pathophysiology are complex. Fortunately, recent advances in cell-specific gene targeting and manipulations in vivo have enabled the in-depth investigation of the signals and mechanisms specifically induced in astrocytes in response to disease. This has uncovered that the contribution of astrocytes to MS and EAE etiopathology is broader than previously believed.

Temporal and anatomical profile of astrocyte reactivity in MS and EAE

In both MS and EAE, astrocyte reactivity is a widespread phenomenon that initiates at the early stage of lesion formation and persists into the chronic phases of lesion evolution, even after immune cell presence has receded. In MS, activated astrocytes with elevated GFAP expression are found throughout the CNS. Their morphology and distribution vary in relation to disease and lesion stage, as well as their positioning in the white or gray matter, suggesting they play different roles. In early pre-lesions, identified by focal mild perivascular lymphocyte infiltration and BBB breakdown, astrocytes are already hypertrophic [101]. In acute active lesions, both in the white and gray matter, astrocytes display a highly reactive phenotype with hypertrophic cell body and thick processes [32]. This phenotype extends into the adjacent normal appearing white and gray matter [32, 101], suggesting reactive astrocytes are early and active contributors

to lesion development. A proportion of the hypertrophic astrocytes observed at the leading edge of actively demyelinating MS lesions show intracellular lipid inclusions. This is indicative of their participation in myelin debris phagocytosis that has been implicated in sustaining lesion pathology [144]. Within active lesions, it is also common to observe reactive astrocytes in close connection with the vasculature displaying signs of damage, with swollen cell bodies and disrupted astrocytic end-feet. This results in extensive gaps in the glia limitans and, consequently, in BBB dysfunction, which allows for increased CNS immune cell entry [32]. On this basis, there is growing support for the idea that the early pathogenic events of MS are dependent, at least in part, on the loss of astrocytic homeostatic function, rather than linked to excessive detrimental astrocytic activation [32]. In chronic lesions, astrocytes appear still reactive, however, their GFAP expression is not as elevated as in acute lesions and their morphology is less rounded and with thinner processes [32]. They are concentrated at the lesion rim. The disruption of the glia limitans still remains, though astrocyte end-feet show reduced swelling and appear connected to the basal lamina [32]. As chronic lesions become inactive, reactive astrocytes also show accumulation of $GFAP^+$ filaments, indicative of the progressive formation of an astroglial scar [32, 101]. Even though more subtle, changes occur also in the white and gray matter adjacent to MS lesions, defined "normal appearing" because of seemingly absent pathology. Here, astrocytes also show signs of activation, although to a lesser extent than within lesions. Overall, regardless of whether astroglial reactivity relates to a loss of homeostatic functions or acquisition of an inflammatory phenotype, the general consensus is that it correlates with disease pathology from the early stages and is a reliable indicator of disease evolution [32].

As far as the type of reactive astrocytes populating the lesion environment, a recent study reported them to be predominantly of the A1 type, which are considered highly pro-inflammatory and neurotoxic [93]. In demyelinating MS lesions, A1 astrocytes, identified by expression of complement component 3 (C3), are observed in close association with $CD68^+$ activated microglia and/or macrophages, which are believed to be potent inducers of the A1 astroglial phenotype. Their number is significantly higher in acute active lesions compared to chronic active and inactive [93].

In EAE, astrocyte activation (assessed by increased GFAP immunoreactivity or gene expression, cellular hypertrophy, thickening of cell processes) is also observed from the early stages of disease, both in the white and gray matter, starting before the appearance of clinical symptoms (locomotor deficits), thus preceding CNS immune infiltration [27, 49, 102, 141, 168, 193]. Astroglial reactivity progressively increases from the pre-symptomatic stage, is maximal at peak/acute disease, particularly in the vicinity of immune cell infiltrates, and persists chronically, though to a lower extent [29, 66, 86, 168]. Astrogliosis, measured in the brain and spinal cord of EAE-induced GFAP-luc mice expressing luciferase under the control of the GFAP promoter, can be assessed as early as 3 days post induction (dpi) of EAE. Importantly, it correlates with and predicts the severity of the EAE clinical course [102]. In mice that develop spontaneous relapsing–remitting EAE (mutated to carry a TCR specific for $MOG_{92–106}$ peptide), reactive perivascular astrocytes with elevated GFAP expression are found well before onset of symptoms in the absence of immune infiltrates [6]. Astrogliosis progresses as infiltration increases, manifesting more prominently on perivascular end-feet and parenchymal astrocytes [6].

In addition to brain and spinal cord, astrocyte reactivity is evident in the optic nerve and retina of EAE affected mice that develop optic neuritis [27, 193]. In the optic nerve, astroglial activation occurs early on in disease, prior to immune cell infiltration, and has been associated with early signs of axonal injury [27, 193]. In our own observations, here astrogliosis remains elevated through the chronic stages of optic neuritis (Fig. 1) (40 dpi of $MOG_{35–55}$ EAE), when axonal degeneration is severe and widespread, as shown by the accumulation of axonal retraction bulbs positive for dephosphorylated neurofilament (SMI32) (Fig. 1). At the same time point (40 dpi), we observe astroglial activation in the retina as well, associated with retinal swelling and disruption (Fig. 2b, yellow arrows) compared to naive mice (Fig. 2a). Here, astrocytes maintain elevated GFAP expression (Fig. 2b) and a concomitantly reduced AQP4 expression at the end-feet (Fig. 2d, white arrows) compared to naïve mice (Fig. 2c). We interpret this as a sign of astroglial damage, whereby astrocytes lacking AQP4 may have a compromised capacity to maintain water homeostasis and BBB functionality.

Similarly to MS, there is evidence that astrocytes suffer cell damage at the early stages of EAE as well. Electron microscopy studies in Lewis rats demonstrated the presence of swollen astrocytes with dispersed GFAP filaments in areas of the spinal cord yet to be infiltrated with immune cells [49]. These structural changes were correlated with increased permeability of the vascular walls, suggesting that astroglial alterations could in turn participate in the progressive disruption of the BBB at the basis of immune cell entry [49]. In our own observations at pre-symptomatic $MOG_{35–55}$ EAE (10 dpi), just before onset, astrocytic activation is already elevated in the spinal cord with increased GFAP immunoreactivity and hypertrophy (Fig. 3). However, we do not find evident signs of structural damage. The astrocytic end-feed maintain AQ4 expression, and the glia limitans appears uninterrupted, suggesting no obvious disruption of the BBB (Fig. 3, white arrows). The discrepant findings may be attributed to various factors, including the different

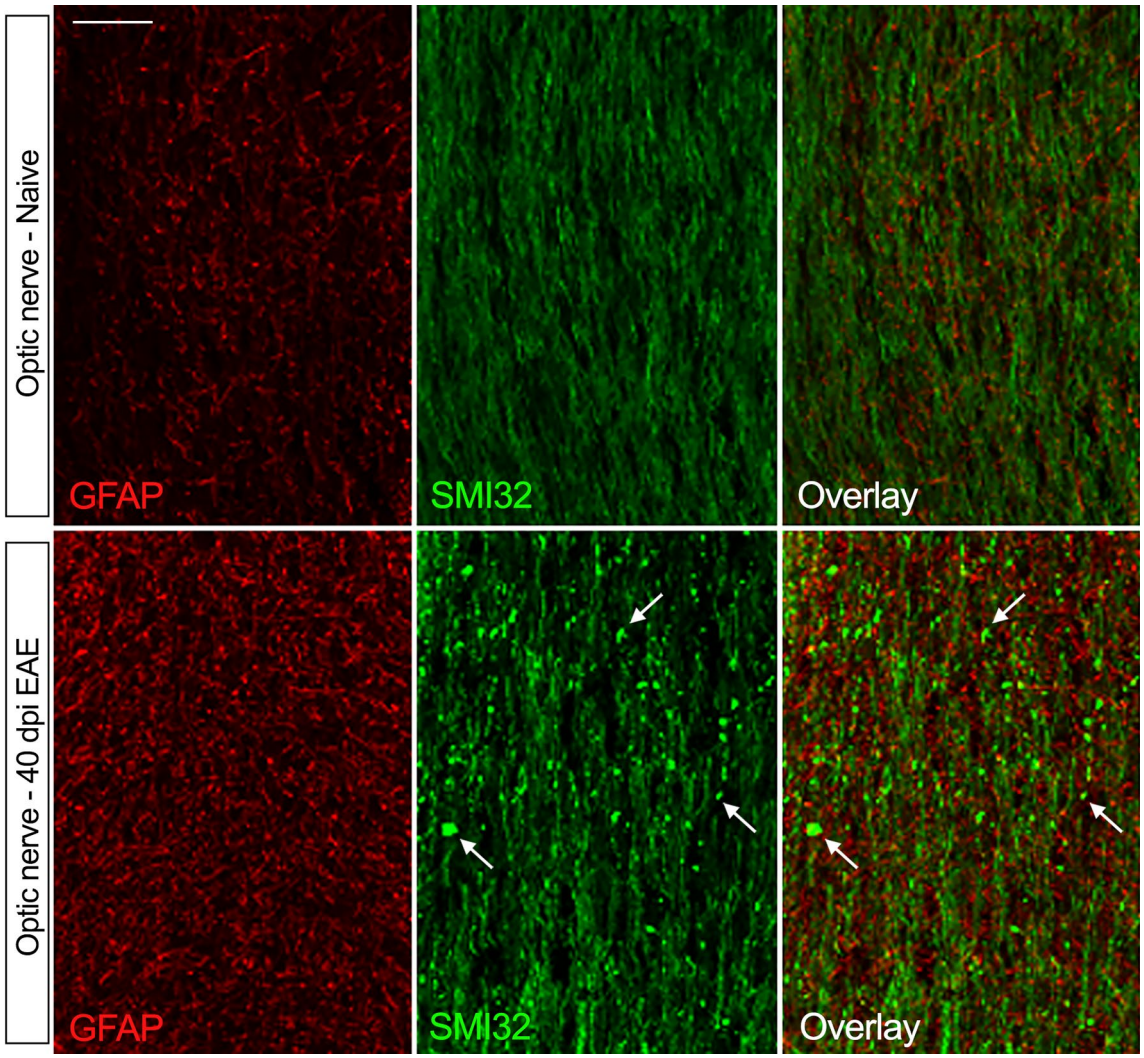

Fig. 1 Astroglial reactivity and axonal damage in the mouse optic nerve at the chronic stage of EAE. EAE was induced by $MOG_{35–55}$ immunization in C57BL/6 mice. Optic nerves of mice sacrificed at chronic disease (40 dpi) were compared to naïve optic nerves. Astrocytes were immunostained for GFAP, and axons for non-phosphorylated neurofilament H (clone SMI32). In naïve conditions, GFAP expression was low, indicative of negligible astroglial activation. In parallel, SMI32 expression, which is elevated in correlation with axonal damage, was mild and diffuse to indicate axonal integrity. At chronic EAE, GFAP was highly upregulated demonstrating increased astroglial activation. This was accompanied by increased reactivity for SMI32, which concentrated in axon retraction bulbs (white arrows), demonstrating ongoing wallerian degeneration of axons. Scale bar: 20 µm

models used and the time and location where astroglial activation was assessed.

Various studies have addressed the significance and main functional contribution of reactive astrocytes during the various phases of disease development and progression using ablation strategies. Selective depletion of proliferating reactive astrocytes achieved with administration of ganciclovir to GFAP-HSV-TK mice resulted in different clinical outcomes depending on the time of depletion. When depletion was initiated prior to EAE induction and protracted up to acute disease, the EAE clinical outcome was markedly exacerbated and associated with increased CNS immune cell infiltration [113, 191]. Similarly, reactive astrocyte depletion at onset with a mild 7-day ganciclovir cycle caused worsening of EAE associated with increased macrophage infiltration and upregulation of CNS inflammatory gene expression [180]. This may be attributed to the loss of astrocytic barrier function, which normally contains immune cells in the perivascular space preventing their entry into the CNS parenchyma [180, 191]. Earlier studies in EAE using GFAP knockout mice reached similar conclusions, with worsening of the EAE course dependent on uncontrolled immune cell ingress into the CNS [94]. Conversely, reactive astrocyte depletion with ganciclovir at the chronic disease phase (starting at 30 dpi) caused improvement of the EAE clinical outcome

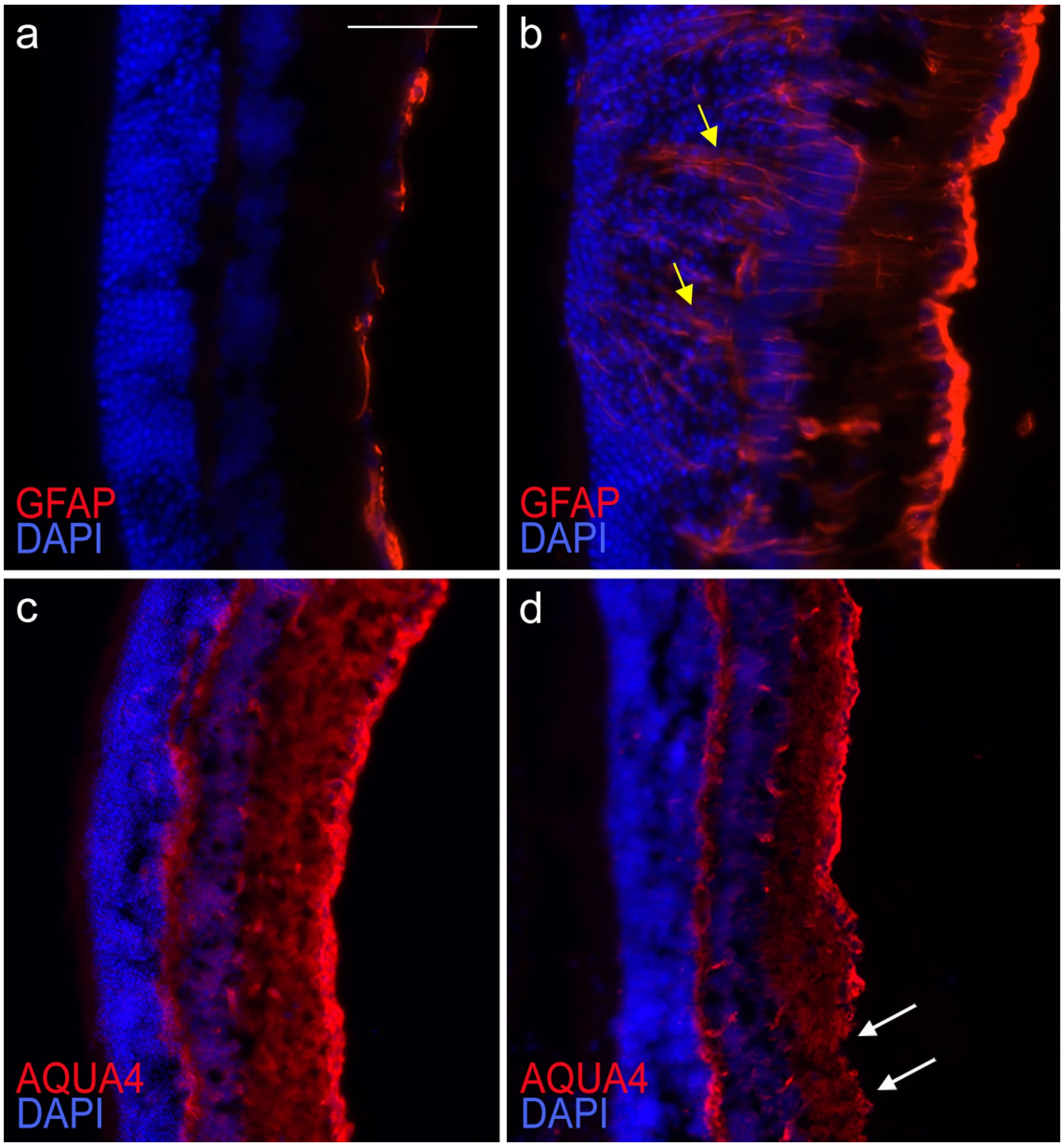

Fig. 2 Astroglial reactivity in the mouse retina at the chronic stage of EAE. EAE was induced by $MOG_{35–55}$ immunization in C57BL/6 mice. Retinas of mice sacrificed at chronic disease (40 dpi) (**b**, **d**) were compared to naïve retinas (**a**, **c**). Astrocytes were immunostained for GFAP (**a**, **b**), or AQP4 (**c**, **d**). Nuclei of retinal ganglion cells (RGCs) were identified by DAPI staining. In naïve conditions, GFAP expression was low (**a**), and AQP4 diffuse and uniformly distributed (**c**), indicative of negligible astroglial activation and intact glia limitans. The RGC layer was compact and uninterrupted (**a**, **c**). At chronic EAE, GFAP was highly upregulated indicating ongoing astroglial activation (**b**). Labelling was evident throughout the RGC layer (**b**, yellow arrows), which appeared disrupted. In parallel, AQP4 labeling was reduced and redistributed, absent in certain areas (**d**, white arrows), suggesting alterations of the glia limitans and potential BBB damage. Scale bar: 50 µm

and was associated with decreased leukocyte infiltration into the CNS [113]. Collectively, these data suggest that, early on in disease, reactive astrocytes play a predominant role in maintaining barrier function against uncontrolled cell trafficking, thereby netting a protective effect, whereas chronically, their contribution to the detrimental neuroinflammatory response is preponderant, thereby netting a detrimental effect. This should be taken into consideration in devising possible therapeutic strategies targeting astroglial activation.

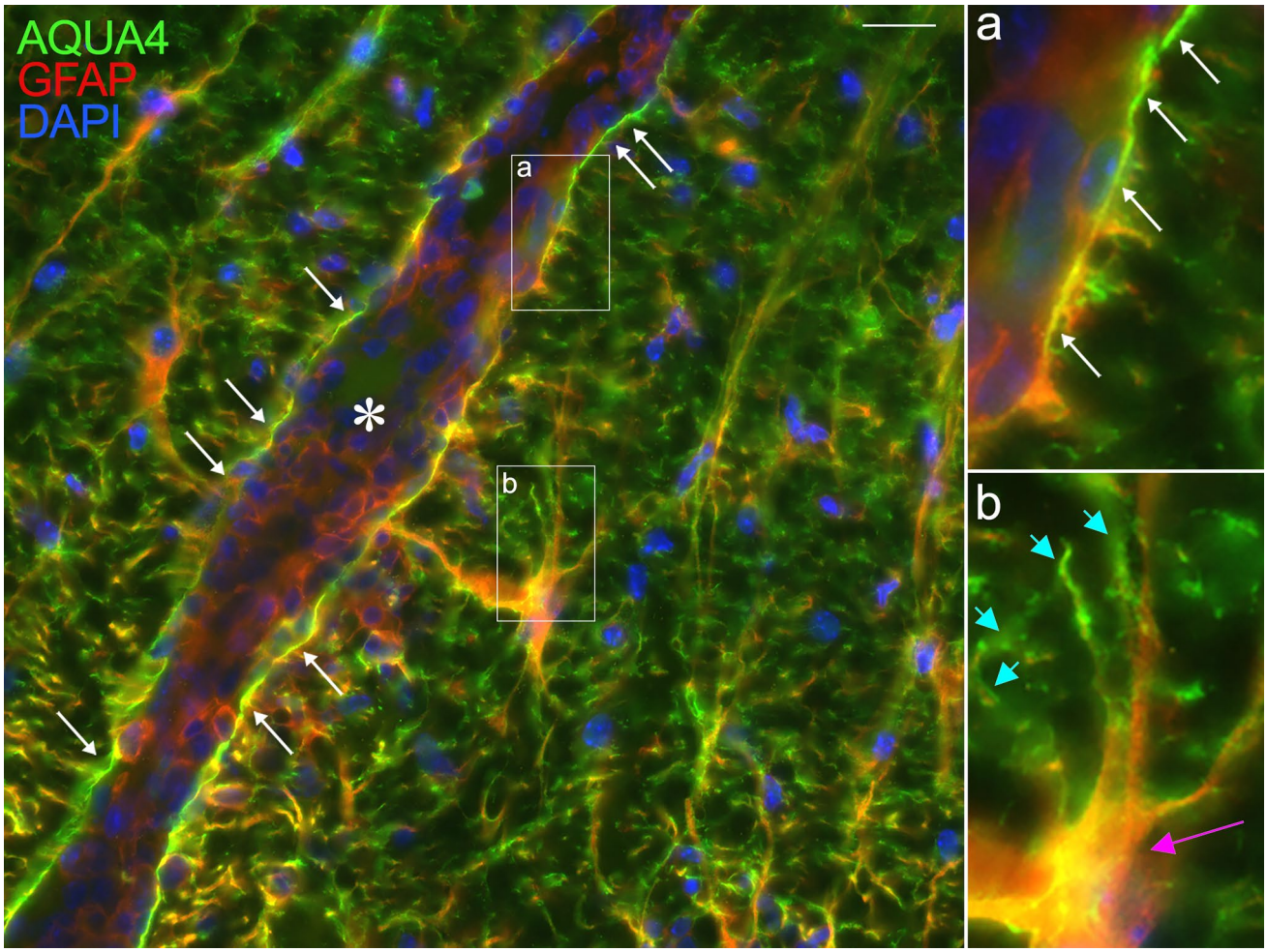

Fig. 3 Astroglial reactivity in the mouse spinal cord at the pre-symptomatic stage of EAE. EAE was induced by $MOG_{35–55}$ immunization in C57BL/6 mice. In spinal cord sections of mice sacrificed at 10 dpi, before onset of symptoms, astrocytes were immunostained for GFAP and AQP4. Strong GFAP labeling was observed, indicative of astroglial activation already occurring. Intense labeling for AQP4 was also observed at the astrocyte end-feet (**b**, blue arrows) as well as on the cell body of hypertrophic astrocytes highly expressing GFAP (**b**, pink arrow). AQP4 and GFAP clearly colocalized within the glia limitans surrounding the blood vessels (**a**, white arrows), where no gaps in AQP4 labeling were observed, indicating that the structural integrity of the glia limitans was maintained at this early EAE stage. *Blood vessel. Scale bar: 10 µm

Signals regulating astrocyte reactivity in MS and EAE

Recent advances in cell-specific gene profiling methods have allowed to gain new insight into the signals driving or suppressing astroglial reactivity in neuroimmune disease. The glycolipid lactosylceramide (LacCer) has been identified as an autocrine signal synthesized in astrocytes by β-1,4-galactosyltransferase 6 (B4GALT6) that promotes pro-inflammatory astroglial activation in EAE [113]. LacCer and B4GALT6 are upregulated in astrocytes during EAE, particularly during the chronic phase. After release, LacCer directs astrocytes towards transcriptional programs that lead to neurodegeneration [113]. Specifically, astroglial LacCer signaling induces the recruitment and activation of microglia and macrophages via production of the chemokine CCL2 and the growth factor granulocyte–macrophage colony-stimulating factor (GM-CSF). Inhibition of B4GALT6 in vivo during EAE abrogates astroglial activation and suppresses the induction of their inflammatory program. This evidence, together with the finding that B4GALT6 is upregulated in hypertrophic reactive astrocytes in MS lesions, suggests that the astroglial LacCer–B4GALT6 axis may be a promising target for MS therapy [113]. Further analyses determined that LacCer operates in astrocytes through activation of the NF-κB and IRF-1 pathways [113]. A recent study showed that, during EAE, also TGFα and VEGF-B induce pro-inflammatory astroglial reactivity via NF-κB activation [152]. These reports are in line with previous studies that identified NF-κB as a primary driver of pro-inflammatory

astroglial activation in EAE [28, 29] as well as other neurological disorders [26]. A more detailed account of astroglial responses dependent on NF-κB is provided in a following section (pathophysiological roles of activated astrocytes in MS and EAE) where cytokine signaling in astrocytes is discussed.

Another key signaling pathway implicated in astroglial activation in CNS autoimmunity is the sphingosine 1-phosphate (S1P) receptor pathway [42, 153]. S1P receptors have been studied mostly in the immune system, as B and T cells have long been considered the target of the disease-modifying drug FTY720 (fingolimod), which is recognized to function by sequestering lymphocytes inside lymph nodes, preventing them from entering the CNS, via binding to S1P receptors. Nevertheless, S1P receptors are also expressed by astrocytes, and their contribution to FTY720 therapeutic effect is now starting to be elucidated. In MS, $S1P_1$ and $S1P_3$ receptors are strongly upregulated on hypertrophic astrocytes in active and inactive white matter lesions [184]. Similarly to MS, administration of FTY720 to EAE-induced mice improves the clinical outcome [42, 153]. This has been shown to be dependent, at least in part, on inhibition of S1P receptor signaling in astrocytes. Indeed, astroglial-specific ablation of S1P receptor drastically reduces the therapeutic effect of FTY720 in EAE [42]. Gene expression profiling of astrocytes isolated from EAE-induced mice untreated or treated with FTY720 showed that inhibition of S1P receptor signaling directed astrocytes towards a reparative program by suppressing pro-inflammatory astroglial activation (downregulation of TNF, IL6, CCL2, CCL20, CXCL10, etc.), while promoting the expression of anti-inflammatory molecules (e.g., IL33, CXCL12) [153].

Just as astrocytes are exposed to factors that stimulate their cellular reactivity during CNS autoimmune disease, they also respond to signals that suppress astrogliosis and inflammatory activation. Type I interferons (IFN-I) belong to this category of molecules. IFN-I, most notably IFNβ, have been used for decades in MS therapy, with their efficacy primarily attributed to suppression of leukocyte-mediated inflammation. However, it is now clear that glia can also respond to IFN-I that are endogenously produced in the CNS in both physiologic and disease conditions [21]. The cellular responses to IFN-I are mediated by the IFN-alpha receptor (IFNAR), composed of the IFNAR1 and IFNAR2 subunits [21], both of which are expressed by astrocytes. In a study by Rothhammer et al. [154], gene expression profiling of astrocytes isolated from EAE-induced and control mice showed that most genes upregulated in reactive astrocytes at acute EAE (28 dpi) are linked to IFN-I signaling [154]. Silencing of IFNAR1 specifically in astrocytes with a lentivirus-delivered shRNA caused worsening of EAE, uncovering an anti-inflammatory role for IFN-I signaling in astrocytes. This is due to IFN-I-dependent upregulation of the aryl hydrocarbon receptor (AhR) in astrocytes, which suppresses CNS inflammation through SOCS2-dependent inhibition of NF-κB [154]. Upregulation of AhR in astrocytes was also observed in active and chronic active white matter MS lesions. Importantly, this study showed that astroglial AhRs exert their suppressive function when stimulated by tryptophan metabolites specifically generated by the gut microbiota [154]. This highlights the importance of the cross-talk between CNS and microbiome in the maintenance of CNS homeostasis, and how dysregulation of this interaction may contribute to the pathogenesis of MS, establishing a link among environment, metabolism and inflammation.

Astroglial inflammatory activation in EAE is suppressed by estrogens. This occurs entirely through estrogen receptor α (ERα), whose conditional ablation specifically in astrocytes abolished the protective effect of estrogens [175, 176]. The mechanism is related to ERα-dependent silencing of NF-κB signaling [59]. ERα and aromatase, a key enzyme for estrogen synthesis, are highly upregulated in hypertrophic astrocytes found in chronic active and inactive lesions, suggesting that estrogen synthesis and signaling in reactive astrocytes may be part of an endogenous protective mechanism in CNS autoimmunity [100].

Pathophysiological roles of activated astrocytes in MS and EAE

Synthesis and response to immunomodulatory factors

Cytokines In the MS- and EAE-affected CNS, the ability of astrocytes to produce and respond to immunomodulatory cytokines with pro- and anti-inflammatory properties is well documented (Fig. 4, Table 1).

In MS, tumor necrosis factor (TNF) is highly upregulated in astrocytes in active lesions, both acute and chronic, especially in astrocytes localized at the lesion edge [72, 161]. TNF is undetected in astrocytes in chronic silent lesions, where the few TNF^+ cells display a macrophage morphology [161]. This establishes a correlation between astroglial TNF expression and lesion severity. It should be noted that, in addition to astrocytes, TNF is produced by a variety of other cell types during MS/EAE (neurons, microglia, immune cells) and its contribution to disease pathology is complex. Indeed, TNF has been attributed both protective and detrimental roles in the CNS affected by neurological disease [148]. The soluble form of TNF (solTNF) is mostly responsible for pro-inflammatory and pro-apoptotic processes via activation of TNFR1, whereas the membrane-bound form of TNF (memTNF) sustains anti-inflammatory and reparative processes via activation of TNFR2 [25, 148]. Given that astrocytes located within active MS lesions show upregulation of ADAM-17 (also known as TNF alpha converting

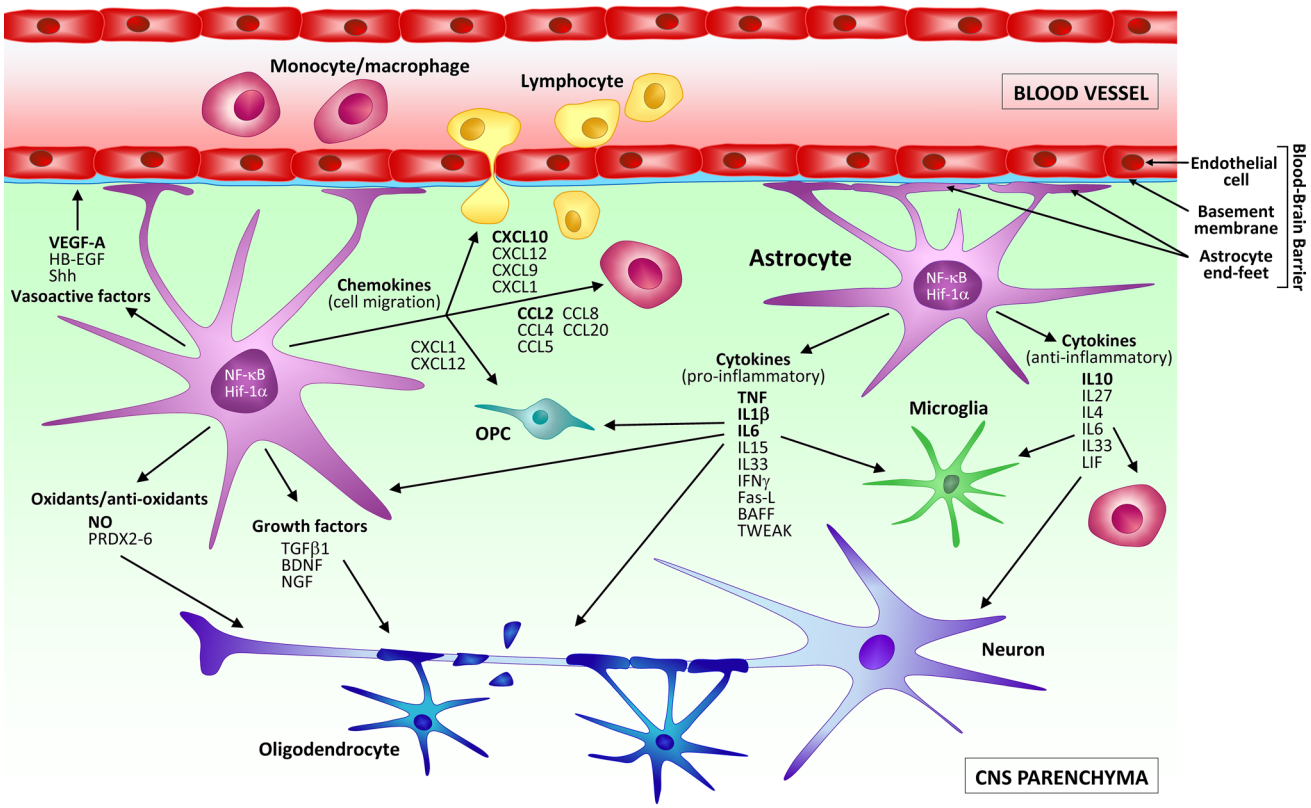

Fig. 4 Schematic representation of the soluble mediators released by astrocytes in MS and EAE. Astrocytes respond to neuroimmune disease with a process of cellular activation that leads to the production of soluble mediators. These signal in a cell autonomous and non-autonomous manner to promote both neurotoxic, and neuroprotective functions. Activated astrocytes release pro-inflammatory cytokines (e.g., TNF, BAFF, TWEAK, IL1β, IL6) that act on immune cells infiltrated in the CNS as well as resident microglia to induce their activation. They also interact with neurons and oligodendrocytes to cause cellular damage. In parallel, astrocytes can also secrete anti-inflammatory cytokines and mediators (e.g., IL10, IL4, IL27, Shh) that behave as immuno-suppressants and contribute to turn off the pro-inflammatory activation of leukocytes and microglia, favouring repair and neuroprotection. Additionally, in MS and EAE astrocytes are a powerful source of chemokines (e.g., CCL2, CXCL10, CCL20, CXCL1), which serve as signals for the continued recruitment of immune cells into the CNS. Select chemokines (e.g., CXCL1 and CXCL12) have been shown to favour the migration of OPCs to the site of demyelination, thus exerting a reparative role. Some of the growth factors released by astrocytes (e.g., VEGF-A) can cause pathological alterations to the BBB, others can promote neuroprotective functions (e.g., BDNF, NGF). Finally, astrocytes participate in the oxidative stress response by producing neurotoxic radicals (e.g., NO), or neutralizing them, specifically via the production of peroxiredoxins (PRDX2-6)

enzyme, TACE), the metalloproteinase responsible for the shedding of pro-inflammatory solTNF [143], it is likely that astrocyte-derived TNF plays a neurotoxic role in MS. On the other hand, reactive astrocytes in MS lesions express TNFR2, the receptor primarily activated by protective memTNF, suggesting that by responding to TNF, astrocytes may participate in repair mechanisms [25]. Following EAE, astrocytes express TNF particularly in and around lesions [188]. Expression peaks at acute disease (12–15 dpi) and is still evident through the chronic disease stage [188]. Astrocytes also respond to TNF during EAE, as they express both TNFR1 and TNFR2 [10]. To date, only a few studies have addressed the function of astroglial TNF receptor-dependent signaling in EAE, as transgenic mice with cell-specific ablation of TNF receptors have only recently become available. Astroglial TNFR1 is responsible for the hippocampal synaptic alterations at the basis of learning/memory impairments observed in EAE. Indeed, Habbas and colleagues demonstrated that increase of TNF in the hippocampus of EAE mice triggers the activation of astrocytic TNFR1 and, in turn, a cascade of astrocyte-dependent neuronal alterations resulting in dysfunction of hippocampal synapses [67]. This could explain, at least in part, the cognitive disturbances associated with EAE and MS. Conversely, astroglial TNFR2 signaling has been attributed protective functions in demyelinating syndromes. Even though this was not investigated in the EAE model of MS, rather in the cuprizone model of demyelination, TNFR2 activation in astrocytes was shown to induce autocrine expression of the pro-myelinating factor CXCL12, which promotes oligodendrocyte precursor cell

Table 1 Astrocyte-derived factors produced in MS and EAE

Molecule	Expression in EAE: model type, time, location	Expression in MS: lesion type, time, location	Function, mechanism
TNF	Various EAE models: acute and chronic disease, in and around lesions [113, 152, 154, 188]	WML: acute active; chronic active (edge); undetected in chronic inactive [72, 161]	Dual effect: pro-inflammatory (solTNF), anti-inflammatory (memTNF)
BAFF	n/a	WML: acute active; chronic active [88]	Pro-inflammatory: B cell survival and expansion (putative)
TWEAK	n/a	WML: chronic active (rim); NAGM: around subpial lesions [163]	Pro-inflammatory (putative)
Fas-L	$MOG_{35–55}$ in C57BL/6 mice: acute disease [195]	n/a	Anti-inflammatory: immunosuppressant by causing T cell apoptosis (putative)
IFNγ	$MOG_{35–55}$ in various C57BL/6 mouse lines [152–154]	n/a	Pro-inflammatory
IL1β	$MOG_{35–55}$ in various C57BL/6 mouse lines [113, 152–154]	n/a	Pro-inflammatory
IL6	$MOG_{35–55}$ in various C57BL/6 mouse lines [54, 113, 152–154]	WML: acute active; chronic active (rim); adjacent parenchyma [13, 159]	Putative dual effect, pro- and anti-inflammatory
IL4	n/a	WML: chronic active (rim), chronic inactive (rim) [76]	Anti-inflammatory
IL10	$MOG_{35–55}$ in various C57BL/6 mouse lines [113, 152–154]	WML: acute active (rim); chronic active (rim) [76]	Anti-inflammatory
IL15	n/a	WML: acute active; chronic active (around vessels and in perivascular cuffs) [155]	Pro-inflammatory: CD8 T cell and NK cell survival and activation (putative)
IL27	n/a	WML: chronic active [162]	Anti-inflammatory
IL33	$MOG_{35–55}$ in C57BL/6 mice: pre-onset, onset, peak disease [41]	WML: unspecified types; perilesional tissue; NAWM [44]	Putative dual effect, pro- and anti-inflammatory
LIF	$MOG_{35–55}$ in various C57BL/6 mouse lines [113, 152–154]	n/a	Anti-inflammatory, pro-remyelination
CCL2 (MCP-1)	Various EAE models in rat and mouse: pre-onset, onset, acute disease [19, 60, 61, 82, 123, 138, 149]	WML: acute active (center); chronic active (rim) [147, 183]; chronic inactive (low) [116]	Pro-inflammatory: immune cell recruitment (macrophages)
CCL4 (MIP-1β)	MBP immunization in Lewis rats: acute disease only [122]	WML: acute active; chronic active [24]	Pro-inflammatory: immune cell recruitment
CCL5 (RANTES)	MBP immunization in Lewis rats: peak disease [122]	WML: acute active; chronic active [24]	Pro-inflammatory: immune cell recruitment
CCL8 (MCP-2)	n/a	WML: acute active; chronic active; chronic inactive (low) [116]	Pro-inflammatory: immune cell recruitment
CCL20 (MIP-3α)	$PLP_{139–151}$ in SJL mice: during relapses [8]; $MOG_{35–55}$ in C57BL/6 mice: high at acute EAE [65]	WML: acute active; chronic active [9]	Pro-inflammatory: immune cell recruitment (dendritic cells, macrophages)
CXCL1 (KC, Gro-1)	$PLP_{139–151}$ in SJL mice: during relapses [60]; $MOG_{35–55}$ in C57BL/6 mice: peak disease [79]	WML: acute active; chronic active [125, 133]	Pro-inflammatory: immune cell recruitment (neutrophils). Protective: OPC recruitment
CXCL9 (MIG)	$MOG_{35–55}$ in C57BL/6 mice; not found in astrocytes, only microglia [37]	WML: acute active; chronic active [166]	Pro-inflammatory: immune cell recruitment (T cells)

Table 1 (continued)

Molecule	Expression in EAE: model type, time, location	Expression in MS: lesion type, time, location	Function, mechanism
CXCL10 (IP10)	Various EAE models in rat and mouse; high at peak [37, 60, 61, 121, 149]	WML: acute active; chronic active; NAWM [17, 166, 173, 174]	Pro-inflammatory: immune cell recruitment (T cells)
CXCL12 (SDF-1α)	$MOG_{35–55}$ in C57BL/6 mice: in basolateral side of endothelium, not clear if in astrocytes [115]	WML: acute active; chronic active (high on glia limitans) [9, 34, 48, 87, 114]	Anti-inflammatory: confines immune cells in perivascular space; OPC recruitment, differentiation (putative)
BDNF	$MOG_{35–55}$ in C57BL/6 mouse lines; acute disease [92, 96]	WML: acute active (high); chronic active (high); chronic inactive (low) [177]	Dual role: neuroprotective and neurotoxic (induces NO production)
HB-EGF	n/a	WML: acute active; chronic active; perilesional tissue [157]	Pro-inflammatory: promotes monocyte trafficking (putative)
NGF	Lewis rats immunized with guinea pig myelin: astrocytes throughout the CNS at acute disease [120]	n/a	Neuroprotective (putative)
TGFβ	$MOG_{35–55}$ in C57BL/6 mice: before and at onset [90, 103]	WML: acute active; chronic active [51]	Pro-inflammatory
VEGF-A	$MOG_{35–55}$ in C57BL/6 mouse lines; high at acute, peak disease in spinal cord lesions [13, 38]	WML: acute active; chronic active [14, 160]	Pro-inflammatory: BBB breakdown and CNS immune cell entry
Shh	$MOG_{35–55}$ in C57BL/6 mouse lines: before onset, highest at acute disease [196]	WML: chronic active (rim); NAWM [5, 196]	Anti-inflammatory: induces endothelial quiescence and improves barrier function
PRDX2	n/a	WML: lesion edge (unspecified stage) [189]	Antioxidant, neuroprotective
PRDX3	n/a	WML: acute active (high); chronic active (low) [127]	Antioxidant, neuroprotective
PRDX5	n/a	WML: acute active; chronic active; chronic inactive; NAWM [74]	Antioxidant, neuroprotective
PRDX6	$MOG_{35–55}$ in C57BL/6 mouse lines: acute disease, near lesions [201]	WML: unspecified stage [201]	Antioxidant, neuroprotective
NO (NOS2 expression)	Various EAE models in mice: acute disease, widespread in and around lesions [45, 154, 182]	WML: acute active (high); chronic active (rim); NAWM [23, 31, 97]	Oxidant, neurotoxic
ROS (activated NOX enzymes)	$MOG_{35–55}$ in C57BL/6 mouse lines: acute disease, in/near lesions [124]	NOX activation not found in astrocytes, only microglia [56]	Oxidant, neurotoxic

Soluble molecules produced by astrocytes during the course of MS and EAE are listed. For EAE, the type of model is reported, and for MS the type of tissue and lesion stage. In parenthesis: corresponding references

n/a not assessed, *WML* white matter lesions, *NAWM* normal appearing white matter, *NAGM* normal appearing gray matter

(OPC) proliferation and differentiation [137]. Collectively, the data available on TNF/TNFR signaling in MS and EAE indicate that astrocytes contribute to maintaining the balance between detrimental and beneficial TNF signaling in the CNS affected by neuroimmune disease.

Other cytokines of the TNF superfamily are produced by astrocytes in MS and EAE. BAFF, which is critical for B cell generation, development and survival, is exclusively expressed by astrocytes in the CNS and is upregulated in MS lesions [88]. In acute lesions, BAFF$^+$ astrocytes are localized around inflammatory cuffs, and in chronic lesions are found in both the perivascular area and the parenchyma where they form a dense network. Interestingly, BAFF$^+$ astrocytes are often in close proximity with immune cells expressing BAFF-receptor (BAFF-R), leading to the speculation that astroglial-derived BAFF promotes the survival of B cells, allowing their persistence and clonal expansion in the MS-affected CNS [88]. Recent studies showed high BAFF levels in the cerebrospinal fluid (CSF) of patients with high cortical lesion load at the time of diagnosis, as well as in the CNS of patients with rapidly progressive disease, suggesting BAFF could be a prognostic biomarker for a severe progressive clinical phenotype [104].

TWEAK, another TNF superfamily member, is found in astrocytes in MS tissues [163]. In chronic active white matter lesions, TWEAK$^+$ astrocytes are localized at the lesion border. In the gray matter, TWEAK$^+$ astrocytes are present in the non-demyelinated gray matter surrounding subpial lesions and in the transitional zone between gray and white matter. The TWEAK receptor Fn14 is also expressed by reactive astrocytes. In the white matter, it is found mainly along the borders of chronic active lesions and in the surrounding normal appearing white matter, and in the gray matter is found both within cortical lesions and in the surrounding normal appearing gray matter [163]. Furthermore, TWEAK and Fn14 are always observed in the areas of worse demyelination and cell damage. Given the pro-inflammatory role of TWEAK-Fn14 in CNS disease, this pattern of expression suggests a pathogenic involvement of astroglial TWEAK signaling in MS [163].

The TNF superfamily member Fas-ligand (Fas-L) is produced by astrocytes during EAE, and it has been attributed a protective role [195]. Studies in mice with astrocyte-specific Fas-L deletion show exacerbation of EAE, with increased immune cell infiltration and pro-inflammatory gene transcription [195]. The protective role of astroglial Fas-L has been associated with its role in inducing T cell apoptosis, thereby suppressing autoimmunity.

Astrocytes are the primary source of IL6 in the MS brain [106, 159]. In acute lesions, IL6$^+$ astrocytes are distributed throughout, whereas in chronic active lesions they accumulate in areas of ongoing inflammation, such as the lesion edges [106]. IL6$^+$ astrocytes are also seen in and around inactive lesions, particularly in the adjacent white matter with oligodendrocyte preservation [106, 159]. It is possible that, since IL6 is known to have a dual effect, pro- and anti-inflammatory, the association of IL6$^+$ astrocytes with oligodendrocytes may signify a protective role for IL6 in myelin repair [159]. Nevertheless, based on current knowledge, the exact function of IL6 in lesion development is not defined. The same can be said for astrocyte-derived IL6 in EAE pathophysiology. Studies in conditional knockouts with astrocyte-specific ablation of IL6 paint a complex picture [54]. While male mice with astroglial IL6 ablation subjected to EAE do not show differences from WT male mice, female mice with astroglial IL6 ablation show a delayed EAE onset compared to female WT mice. This is accompanied by reduced demyelination and astrogliosis at chronic disease, suggesting a possible protective role for astroglial IL6 that appears to be sex-specific [54].

A common feature of TNF, IL6 and numerous other molecules (e.g., chemokines and growth factors discussed below) produced by activated astrocytes during neuroimmune disease, is that their synthesis is primarily controlled by the canonical NF-κB signaling pathway [119]. Because of its target gene signature, NF-κB has long been considered the master transcriptional regulator of inflammation [179]. The importance of NF-κB signaling in sustaining pro-inflammatory astroglial activation in EAE has been demonstrated using transgenic approaches. Mice with astrocyte-specific inhibition of NF-κB via GFAP-driven overexpression of the IκBα super-repressor (GFAP-IκBα-dn mice) show improved EAE with reduction of neuroinflammation assessed by suppression of inflammatory gene expression and immune cell infiltration in the CNS [27–29, 186]. Among the downregulated genes, many are themselves potent NF-κB activators, such as IL1β [29, 69]. This suggests that, via NF-κB, reactive astrocytes respond to EAE by producing inflammatory mediators, including cytokines, which in turn act in a cell autonomous manner and further stimulate their NF-κB-dependent production. It has been shown that this mechanism is kept in check by A20, a ubiquitin-modifying protein which physiologically inhibits NF-κB activation. Indeed, ablation of astrocytic A20 causes an increase in pro-inflammatory cytokine production (including TNF, IL6 and IFNγ) following EAE, resulting in disease exacerbation [194].

Astrocytic production of pro-inflammatory cytokines is also sustained by the T cell-derived cytokines IL17 and IFNγ, which are heavily released in the CNS during the acute phase of EAE. Astrocytes are responsive to IL17 as they physiologically express functional IL17 receptor A (IL17RA), which they upregulate following EAE [50]. Exposure of astrocytes in vitro to IL17 alone or in combination with other cytokines (e.g., TNF and IFNγ) stimulates the production of cytokines and chemokines, and increases the expression of the inducible nitric oxide synthase 2 (NOS2)

[53, 117, 181]. Impairment of the IL17 signaling pathway in astrocytes via ablation of Act-1, an essential component of the IL17R complex, causes suppression of IL17-mediated chemokine expression and is associated with disease amelioration [78]. This indicates that via IL17 astrocytes play an important role in leukocyte recruitment during CNS autoimmunity. One of the mechanisms by which IL17 regulates the production of inflammatory mediators in astrocytes during EAE is the modulation of microRNA expression. With a combination of in vitro and in vivo studies, miR-873 induced by IL17 stimulation in astrocytes was shown to promote cytokine production via suppression of A20, thus lifting the inhibition of pro-inflammatory NF-κB activation [98]. Using a similar approach, miR-497 was also shown to participate in IL17-dependent pro-inflammatory gene expression in astrocytes following EAE. Indeed, activation of IL17 signaling in astrocytes causes a downregulation of miR-497 unleashing expression of the transcription factor Hif-1α and subsequent upregulation of pro-inflammatory genes. Restoring miR-497 to astrocytes of EAE-induced mice improved the clinical outcome, underscoring the relevance of the IL17-miR-497-HIF-1α axis to EAE pathophysiology [164].

Unlike IL17, the reports on the astrocytic response to IFNγ in EAE have been contradictory. Silencing of IFNγ signaling in astrocytes via lentiviral knockdown of IFNγR ameliorated EAE by suppressing the production of cytokines and chemokines, thus limiting CNS immune cell trafficking [52]. Yet, studies where astrocytic IFNγ signaling was suppressed via expression of a signaling-deficient dominant-negative IFNγ receptor 1 in astrocytes (GFAPγR1Δ mice) showed the opposite. Indeed, GFAPγR1Δ transgenic mice displayed remarkably increased EAE severity and progression, with enhanced astrocytic activation, demyelination and axonal damage [71]. These outcomes were linked to elevated expression of astrocytic IL6 and sustained microglial activation [156].

The cytokines IL33 and IL15 have also been detected in astrocytes in MS and EAE, and their function is yet to be understood. IL33, which has been attributed dual pro- and anti-inflammatory roles [44] is observed in astrocytes present both in lesional and perilesional tissue, as well as in the normal appearing white matter of MS brains. Its significance is unknown, although studies in EAE show that neutralization of IL33 actively secreted by astrocytes exacerbates disease [41], pointing at a protective role of astroglial derived IL33 in CNS autoimmunity. High expression of IL15 is detected in reactive astrocytes in MS tissues both in acute and subacute/chronic lesions [155]. $IL15^{+}$ astrocytes are also found around blood vessels with or without perivascular cuffs [155]. Since IL15 is essential for development, activation, and survival of CD8 T lymphocytes and NK cells, it is plausible that production of IL15 is one of the ways by which astrocytes communicate with the adaptive immune system contributing to MS immunopathology [155].

Finally, various cytokines known for their anti-inflammatory and neuroprotective effects are expressed by reactive astrocytes in the MS brain. Strong IL10 immunoreactivity is observed in astrocytes within acute active lesions, as well as at the rim of chronic active lesions [76]. IL4 is detected in astrocytes within the hypocellular rim of chronic active and inactive lesions. [76]. Similarly, the immunosuppressive cytokine IL27 is upregulated in astrocytes within chronic lesions [162]. Importantly, their respective receptors IL10R, IL4R and IL27R are also expressed and upregulated in astrocytes in active and chronic active lesions [76, 162]. These patterns of expression suggest these cytokine systems may be implicated in MS lesion repair, potentially turning off lesion activity.

Chemokines In MS and EAE, astrocytes become a powerful source of chemokines, whose function is to guide cell migration and trafficking (Fig. 4, Table 1). While a handful of these chemokines display protective properties, most of them are chemoattractant for detrimental immune cells, thus their production is generally associated with sustaining and exacerbating the pathology.

In active demyelinating and chronic active MS lesions, reactive hypertrophic astrocytes highly express CCL2 (MCP-1). Immunoreactivity is especially elevated in the center of acute active lesions and within the rim of chronic active lesions, in close association with macrophages [183]. Low astroglial expression is also reported in chronic inactive lesions [116]. In a study looking at white matter versus gray matter lesions in the hippocampus, CCL2 expressing astrocytes were found only in active white matter lesions where macrophages were present and immunoreactive for the CCL2-specific receptor CCR2. Astrocytes did not express CCL2 in gray matter lesions characterized by a paucity of immune cells [147]. Being CCL2 a powerful chemoattractant for monocytes/macrophages, this pattern of expression suggests that reactive astrocytes play a role in the recruitment of myelin-degrading macrophages, thereby specifically contributing to the evolution of white matter MS lesions [147, 183]. This may also explain the low immune cells presence in gray matter lesions where astrocytes do not express CCL2.

Multiple histological studies have localized CXCL10 (IP10) to reactive astrocytes in active demyelinating MS lesions and the surrounding parenchyma [17, 166, 173, 174]. Expression is observed especially around inflammatory infiltrates, both in the astrocyte cell body and end-feet [173]. A similar pattern was reported for CXCL9 (MIG), whose main localization is in astrocytes surrounding the lesions [166]. Importantly, T cells expressing CXCR3, the receptor responsive to both CXCL10 and CXCL9, are found within

the lesions and accumulate as lesions develop [166, 174]. This indicates that astroglial CXCL10/CXCL9 signaling towards CXCR3 expressing T cells is an important mechanism driving T cell accumulation in the CNS perivascular space and parenchyma during MS lesion formation.

CCL2 and CXCL10 were the first two chemokines to be also identified in astrocytes during EAE [19, 37, 60, 61, 149]. For both, astroglial expression was only observed after the onset of clinical signs and was the highest at peak/acute disease, correlating with disease activity [19, 61]. Histological studies showed astrocytes in close proximity with infiltrating leukocytes had the most intense immunoreactivity [61]. This indicates that leukocyte-derived factors are likely the main stimuli triggering astroglial production of CCL2 and CXCL10 in EAE. Recent studies showed that transgenic mice with conditional ablation of CCL2 in astrocytes have less severe EAE, accompanied by reduced macrophage and T cell infiltration, reduced glial reactivity and improved neuroprotection [82, 123, 138]. Similarly, deletion of astroglial CXCL10 resulted in EAE improvement, with delayed onset and mild reduction of severity, despite not being effective in protecting from progressive axon loss [121]. Absence of CXCL10 reduced the ability of $CD4^+$ T cells to enter the CNS parenchyma, accumulating in the perivascular cuffs instead [121]. Similar to the findings in MS, these reports underscore the crucial role astrocyte-derived CCL2 and CXCL10 play in the continued recruitment of immune cells that sustain disease progression.

In MS, astrocytes control CNS monocyte/macrophage trafficking also via CCL5 (RANTES) and CCL8 (MCP-2). These chemokines are upregulated in astrocytes within active and chronic active MS lesions [24, 116]. They both act through the receptor CCR5, which is highly expressed in monocytes (and few lymphocytes) concentrated in active lesions [17]. This profile has been reported in EAE as well, where astrocytes ramp up production of CCL5 as well as CCL4 (MIP-1β) after EAE onset [122]. Astroglial synthesis of CCL5 and CCL4 is initiated in response to T cell-derived IL17 and IL6 [122], indicating that, like CCL2 and CXCL10, leukocytes provide the initiating signals.

Astrocytes are the CNS cells expressing CXCL12 (SDF-1α) in the MS brain. A key mediator of B cell migration, CXCL12 is present in astrocytes and on blood vessels under normal conditions, and its expression is upregulated in MS, both in active and chronic inactive lesions [9, 87, 114]. The ability of astrocytes to produce CXCL12 has been amply demonstrated in vitro after stimulation with inflammatory cytokines [9, 22]. It has been observed that, in active MS lesions, CXCL12 on blood vessels changes distribution, rearranging toward the lumenal side of venules. At the same time, astrocytes increase CXCL12 expression within the glia limitans [114]. This change in CXCL12 polarity has been associated with impairment of the BBB and positively correlated with increased inflammation and demyelination in MS. This suggests that CXCL12 normally functions to localize infiltrating leukocytes to perivascular spaces, preventing their CNS parenchymal infiltration. When CXCL12 polarity is disrupted during MS, this leads to increased leukocyte adherence to vessels, which facilitates their migration into the CNS [114]. Similarly to MS, CXCL12 is upregulated in the CNS after EAE. At acute disease, it is found redistributed to the basolateral side of the CNS endothelium, and this is believed to be a mechanism by which leukocytes are allowed entry into the CNS during EAE [115]. Differently from MS, however, a clear expression in the astrocyte component of the glia limitans was not observed [115]. Beside these detrimental chemoattractant properties, astroglial CXCL12 has also been attributed protective functions. Indeed, it promotes the polarization of effector Th1 cells into $IL10^{high}$ antigen-specific regulatory T cells, thereby restraining the autoimmune process [118], and it may play a role in attracting $CXCR4^+$ OPCs, contributing to remyelination and repair in CNS demyelination [136].

In MS, the chemokine CXCL1 (KC, Gro-1) is found on hypertrophic astrocytes in active lesions in close proximity to proliferating oligodendrocytes expressing CXCR2, the specific receptor for CXCL1. By acting through CXCR2, CXCL1 is a major chemoattractant for diverse cell populations, including neutrophils and OPCs [125]. This may indicate that, via release of CXCL1, reactive astrocytes recruit $CXCR2^+$ OPCs to the sites of demyelination, thereby contributing to myelin repair [133]. Production of CXCL1 by astrocytes during EAE has also been reported [60]. Transgenic mice transiently overexpressing CXCL1 in astrocytes at the onset of EAE show a milder disease phenotype associated with reduced axonal pathology and improved remyelination at chronic stages, despite the acute increase in neutrophil infiltration driven by CXCL1. This indicates that the astroglial–oligodendroglial CXCL1–CXCR2 axis has a powerful neuroprotective function due to the CXCL1-dependent migration of OPCs to the lesion sites where they can initiate remyelination, which is sufficient to counteract the detrimental neutrophil effect [134]. Contrary to this study, a recent report showed that sustained overexpression of astroglial CXCL1 during EAE is detrimental and exacerbates the outcome by worsening inflammation and demyelination. This was dependent on overt and continuous neutrophil infiltration [64]. Interestingly, it has been shown that treatment of EAE-induced mice with ERβ ligands drastically reduced EAE severity. This correlated with increased astroglial production of CXCL1, leading to improved remyelination and neuroprotection [79].

Mild production of CCL20 (MIP3α) has been reported in astrocytes in MS lesions [9]. Expression is observed in EAE as well, where astrocytes are the principal source of CCL20 [8]. Here, astrocytic CCL20 production has been shown to

be triggered by T cell-derived RANKL, a member of the TNF superfamily of cytokines [65]. Indeed, inhibition of RANKL leads to downregulation of CCL20 and results in suppression of EAE [65]. In addition to T cells, CCL20 is a powerful chemoattractant for dendritic cells, macrophages and, to a lesser extent, neutrophils. This may indicate a role for astroglial CCL20 in modulating the innate immune response in MS and EAE.

Growth factors Astrocytic production of a variety of growth factors has been demonstrated both in MS and EAE (Fig. 4, Table 1). Brain derived neurotrophic factor (BDNF) is found in reactive astrocytes within active and, to a lesser extent, inactive MS lesions [177]. It is also present in EAE lesions at the onset/acute stage of disease (14 dpi) [96]. Here, BDNF has been attributed neuroprotective functions. Indeed, genetic ablation of BDNF specifically in astrocytes exacerbated EAE and increased axonal damage [96], despite not altering CNS immune cell infiltration. An identical profile was observed when astroglial BDNF was inducibly ablated after EAE induction during the preclinical phase (up to 11 dpi) [92]. Studies with bone marrow chimeras showed this neuroprotective effect is solely attributed to CNS-derived and not immune cell-derived BDNF, uncovering an important reparative function for activated astrocytes in CNS autoimmunity [92]. Astrocytes not only produce BDNF, but also respond to it via activation of TrkB receptors. TrkB is markedly upregulated on astrocytes during EAE, as well as in MS chronic inactive lesions [45]. Surprisingly, astroglial TrkB activation is neurotoxic in EAE, as transgenic mice with astrocyte-specific TrkB ablation show reduced neuronal damage. This is due to NO released by astrocytes as a result of BDNF-dependent TrkB activation [45]. Together, these studies indicate that astroglial BDNF not only elicits neuroprotective effects in other cell types, but can also cell autonomously activate astrocytes to release toxic NO, thus causing neurodegeneration. This suggests a possible dual protective and degenerative role for astroglial BDNF in neuroimmune disease.

Astroglial expression of all the transforming growth factor beta (TGFβ) isoforms has been detected in MS lesions at various stages, from active demyelinating to chronic active and inactive [51], suggesting a role in lesion development and evolution. In EAE, TGFβ1, in particular, was found to be upregulated in astrocytes before and during the onset phase of EAE [90, 103]. This has been shown to depend on astroglial production of angiotensin II, which can in turn stimulate astrocytes to produce TGFβ1 in a positive feed-back loop [90]. Both angiotensin II and its precursor angiotensinogen have been described in reactive perivascular astrocytes in MS lesions, thus indicating that a similar mechanism of TGFβ1 regulation may be relevant to MS pathogenesis as well. Blockade of TGFβ1 upregulation with the angiotensin II type 1 receptors (AT1Rs) inhibitor candesartan improved the clinical course of EAE and reduced lymphocyte infiltration, supporting a detrimental role for astroglial TGFβ1 in EAE [90]. This is further corroborated by studies with transgenic mice overexpressing astroglial TGFβ1, which, after EAE challenge, show earlier onset and increased disease severity [198]. Notably, the detrimental effects of astroglial-derived TGFβ1 are in contrast with peripherally produced or administered TGFβ1, which has been attributed protective functions in EAE due to its immuno-suppressive and anti-inflammatory properties [68, 77].

Astrocytic expression of the angiogenic vascular endothelial growth factor A (VEGF-A) has been reported in active demyelinating and chronic MS lesions [14, 160], particularly in highly reactive astrocytes around blood vessels, and in EAE [13]. Astroglial VEGF-A is a key driver of BBB permeability in neuroimmune disease [13]. Indeed, transgenic ablation of astrocytic VEGF-A reduced BBB breakdown by preventing the disruption of endothelial claudin-5 (CLD5) and occludin (OCLN), thus preserving tight junctions. This limited lymphocyte infiltration, resulting in reduced paralysis and neuroprotection [13]. A primary signal directing the expression of VEGF-A during developmental angiogenesis is the transcription factor Hif-1α. Since Hif-1α and VEGF-A are highly expressed and colocalize to astrocytes in MS lesions, it has been suggested that reactivation of this developmental angiogenic pathway in astrocytes is responsible for BBB breakdown during CNS autoimmunity [14]. Furthermore, astroglial VEGF-A has been shown to synergize with astrocyte-produced thymidine phosphorylase (TYMP) to induce BBB disruption. Indeed, blockade of either one or both factors suppresses EAE and improves BBB function [38].

Reactive astrocytes in active and chronic active MS lesions, as well as perilesional tissue, highly express heparin-binding epidermal growth factor (HB-EGF) [158], a trophic and chemotactic factor induced by inflammatory stimuli. Expression is also observed on astrocyte end-feet wrapping blood vessels. Though its role in CNS autoimmunity is unclear, since blockade of HB-EGF reduced the migration of monocytes across brain endothelial cell monolayers, it is plausible to hypothesize it may be implicated in immune cell trafficking across the BBB, thus participating in lesion development and evolution [158].

Astroglial expression of nerve growth factor (NGF) has been observed throughout the CNS (spinal cord, corpus callosum, cortex, brain stem) during the acute stage of EAE in rats [120]. Whether astrocytes are a source of NGF also in the MS-affected CNS has not been reported. NGF function in neurodegenerative disorders is still a matter of debate, as this factor has been attributed both protective and detrimental effects on neuronal and OPC survival. One hypothesis is

that enhanced NGF production may be an attempt at activating protective signals to counteract neuronal damage and promote oligodendrocyte survival [120].

Modulation of oxidative stress

Oxidative stress is the complex cascade of deleterious events induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS). It occurs when the antioxidative system is overwhelmed, particularly during pathological conditions. ROS are mostly byproducts of mitochondrial oxidative phosphorylation, generated via the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (or NOX enzymes). The main RNS is the free radical NO, generated by nitric oxide synthases. In the CNS, astrocytes are major players in the antioxidative system. Indeed, they have the capacity to produce ROS and RNS, but are also the main cell type responsible for their neutralization through glutathione and ascorbic acid, as well as detoxifying enzymes, such as superoxide dismutases (SODs) [187]. During MS and EAE, ROS and RNS are produced by astrocytes, as well as other CNS and immune cells, and they have been implicated in mechanisms underlying lesion pathogenesis (Fig. 4, Table 1).

In MS, extensive oxidative damage to lipids, proteins, and nucleic acids is observed throughout active demyelinating and chronic active lesions, especially within reactive astrocytes and myelin-laden macrophages [185]. Interestingly, at the same time astrocytes markedly upregulate the expression of the antioxidant enzymes superoxide dismutase 2 (SOD2) and NAD(P)H:quinone oxidoreductase 1 (NQO1), which may reflect an adaptive defense mechanism to reduce ROS-mediated cellular damage [185]. Astrocyte can counteract oxidative damage also via the activity of peroxiredoxins (PRDXs), which catalyze the reduction of peroxides to alcohols. In MS, PRDX2 is found upregulated in astrocytes in white matter lesions, particularly at the edges [189]. Expression levels positively correlate with the degree of inflammation and oxidative stress [189]. PRDX3 is detected strongly in acute active lesions and to a lesser extent in chronic active lesions [127]. PRDX5 is found in astrocytes in acute, chronic active and inactive MS lesions, as well as in the normal appearing white matter [74]. PRDX6 is also found in astrocytes within lesions, both in MS and EAE [201]. In EAE, transgenic mice overexpressing PRDX6 showed expression almost exclusively localized to astrocytes, mainly near demyelinating lesions. Increased levels of astroglial-derived PRDX6 correlated with a reduction in EAE clinical symptoms associated with diminished inflammation and CNS damage [201]. Collectively, these data indicate that, like SOD2 and NQO1 expression, PRDXs expression may function as an adaptive reaction of astrocytes to contain oxidative damage.

On the opposite side, astrocyte cause oxidative stress by producing NO. Intense upregulation of inducible nitric oxide synthase 2 (NOS2), as well as of its catalytic activity, is detected in hypertrophic astrocytes throughout active demyelinating lesions and in the adjacent normal appearing white matter [23, 31, 97]. In chronic active lesions, astroglial NOS2 expression is also evident, particularly at the lesion edge [23, 31, 97]. This indicates that astrocyte-derived NO produced via NOS2 may contribute to cytotoxicity mediated by oxidative damage. Astrocytes are also a major source of NO during EAE. Indeed NOS2 expressing astrocytes are found near inflammatory infiltrates in the spinal cord, as well as the adjacent white matter [182]. Astroglial expression of NOS2 has also been confirmed in recent transcriptomic studies [154]. BDNF has been identified as one of the signals driving astroglial NOS2 upregulation in EAE via TrkB stimulation and subsequent activation of the NF-κB pathway [154].

In EAE, astrocytes may contribute to oxidative stress also via the hyperactivation of NOX enzymes. NOX enzyme overactivation is detected in astrocytes in acute EAE lesions and is associated with accumulation of immune cells and damage [124]. This seem to contrast with MS lesions, where upregulation of NOX enzymes is observed only in microglia and macrophages [56].

Maintenance of BBB integrity and function

Blood vessels, glia and neurons act as an integrated neurovascular unit that controls the functionality of the BBB [1]. Astrocytes, whose end-feet virtually wrap the entire vascular surface, are key structural elements of the BBB directing the bidirectional transit of molecules and cells between blood and CNS through multiple mechanisms. Their dysregulation results in BBB dysfunction and is a typical feature of neurological diseases including MS, where focal disturbances in the BBB have been associated with the formation of inflammatory lesions [7, 190].

In MS tissues, intense astrogliosis with increased numbers of $GFAP^+$ end-feet is detected around blood vessels at the center of active lesions [4]. This is associated with degradation of endothelial junctional proteins (e.g., OCLN, VE-cadherin) which are necessary for proper BBB functionality [4]. This suggests that signals provided by activated astrocytes likely participate in BBB disruption and facilitate the entry of immune cells promoting lesion formation [4]. Furthermore, within active lesions, reactive astrocytes show severe disruption of the end-feet connecting them to the vasculature. This leads to gaps in the glia limitans and loss of BBB structural integrity, allowing for CNS immune cell entry [32]. Direct astrocyte–astrocyte and

astrocyte–endothelial cell communication through gap junctions is also key to maintaining BBB function. Impairment of astrocytic gap junctions has been observed in MS lesions, both actively demyelinating and chronic active, with reduction in the expression of Cx43, their main structural component [111]. This has been linked to BBB disruption, as Cx43 loss leads to astrocyte end-feet edema causing detachment from the basal lamina and weakening of the BBB [55]. Severe loss of astrocytic Cx43 has been especially observed in actively demyelinating lesions of rapidly progressing MS cases who experienced higher relapse rates [112], whereas milder MS phenotypes did not show appreciable changes in astroglial Cx43. In the severe cases, the diffuse loss of Cx43 was also accompanied by a patchy loss of the water channel protein AQP4 at the astrocyte end-feet. Since this is also an integral component of the BBB structure, it is plausible that concomitant Cx43 and AQP4 astrocytopathy contributes to a higher degree of BBB damage that leads to unrestricted immune cell infiltration thus fulminant MS progression [112]. In other reports, reduced astrocytic Cx43 was observed in chronic inactive white matter lesions, whereas a modest increase was found in gray matter lesions and normal appearing gray matter [108, 109]. Rather than associated with BBB dysfunction, this increase has been interpreted as an indicator of astrogliosis causing aberrant astrocytic intercommunication that could be at the basis of cognitive impairments in MS patients [108, 109]. A systematic characterization of AQP4 expression and distribution in MS tissue was undertaken by Sinclair and colleagues, who found an increase in all categories of MS tissue, with the highest levels in active lesions [167]. Such increase was most pronounced at the astrocytic end-feet but was also found in the cell bodies of parenchymal astrocytes [167]. This suggests that aberrant AQP4 function at the BBB may be involved in the initiation of the diffuse vasogenic edema observed in MS [167].

In EAE, similarly to MS, BBB impairment due to altered astroglial AQP4 function has been associated with vasogenic edema and tissue damage [197]. Indeed, in the spinal cord and cerebellum of EAE-induced mice AQP4 was found to be disorganized and redistributed over the entire cell surface rather than confined to the end-feet. This loss of polarized AQP4 localization caused altered water redistribution across the BBB and edema formation [197]. A key protein in maintaining proper AQP4 organization, thus structural integrity of astrocyte end-feet and BBB, is dystroglycan [128, 197]. On one side, dystroglycan connects the astroglial end-feet to the endothelial basement membrane, on the other it is anchored to the astroglial cytoskeletal machinery. During EAE, loss of astrocytic dystroglycan due to cleavage by the metalloproteases MMP2 and MMP9 causes breakdown of the endothelial basement membrane and focal leakiness of the BBB, allowing unrestricted ingress of $CD4^+$ T cells in the CNS parenchyma [3]. Recently, it has been reported that during CNS inflammation reactive astrocytes form de novo structural tight junction complexes within the end-feet in response to pro-inflammatory signals [75]. These contain claudin-1 (CLDN1), claudin-4 (CLDN4), and junctional adhesion molecule A (JAM-A). The newly formed tight junctions increase the impenetrability of the glia limitans component of the BBB, corralling T cells into segregated clusters and preventing their entry into the CNS [75]. Conditional ablation of CLDN4 specifically in astrocytes disrupts the formation of these protective tight junctions, leading to unchecked leukocyte infiltration and worsening of EAE [75]. Collectively, these studies underscore how the structural integrity of astrocytes is pivotal in maintaining the architecture and functionality of the BBB during neuroimmune disease. This is further supported by the observation that, in pre-symptomatic EAE, damaged swollen astrocytes with dispersed GFAP filaments are found in areas with increased vascular permeability, but yet to be infiltrated with immune cells [42]. This indicates that loss of astroglial cyto-integrity is a prerequisite for BBB disruption at the basis of immune cell entry [42].

Another important task operated by astrocytes at the BBB is the regulation of iron homeostasis. Toxic ferrous iron accumulates in disease and its detoxification requires conversion into ferric iron that can be dumped into circulation through the astrocyte end-feet in contact with the vasculature. In EAE, astrocytes near the site of lesions upregulate expression of iron importers, suggesting they possess mechanisms to actively uptake iron during disease progression [202]. Astrocytes also express iron exporters at all stages of EAE, indicating they are capable of effluxing iron and safely recycle it out of the CNS. These mechanisms likely explain why iron does not accumulate in astrocytes during EAE. The robust clearance of iron by astrocytes might also contribute to the lower level of iron buildup observed in the EAE spinal cord [202].

Astrocytes modulate BBB function also through the synthesis and release of soluble factors. In addition to those described in the paragraphs above (e.g., cytokines, VEGF-A, NO), sonic hedgehog (Shh) must be mentioned for its protective effect on barrier function. Shh is highly upregulated by reactive perivascular astrocytes in MS lesions [5, 196]. Using in vitro/in vivo approaches, astrocyte-derived Shh was shown to act on barrier-forming endothelial cells promoting their quiescence. Indeed, it downregulated their expression of intercellular adhesion molecule-1 (ICAM-1) as well as of the chemokine CCL2, thereby reducing the adhesion and migration potential of T cells across the BBB [5]. Furthermore, astroglial Shh reduced the activation state of Th1 and Th17 cells, dampening their production of pro-inflammatory molecules [5]. On this basis, it is no surprise that pharmacological inhibition of the Shh pathway caused

significant EAE exacerbation, associated with increased immune cell presence in the CNS and worse demyelination. This underscores the crucial role of astroglial Shh in maintaining proper barrier function and sustaining the astroglial-specific anti-inflammatory response during CNS immune attack.

In vivo measurements of astroglial reactivity: diagnostic and/or prognostic tools for MS?

In MS, non-invasive imaging techniques are essential to aid diagnosis, monitor disease progression and treatment efficacy, as well as uncover underlying pathogenic mechanisms. Though magnetic resonance imaging (MRI) has been the gold standard in MS diagnosis and monitoring, its ability to assess functional processes and gain information on disease-associated molecular events is limited. To the contrary, this is possible with positron emission tomography (PET), which is now emerging as a powerful tool for in vivo functional imaging. It allows quantification of cellular targets that can help understand mechanisms underpinning MS even prior to the occurrence of structural changes detectable by MRI. Neuroinflammation can be effectively assessed by PET, and astroglial reactivity is one of its hallmarks that can be targeted for this purpose. Since a large body of evidence has now established a positive correlation between astroglial reactivity and disease progression in MS [32], PET imaging of this phenomenon is an appealing non-invasive method to potentially enhance early and accurate diagnosis for precision therapy. PET imaging of astroglial reactivity is still in its infancy and in need for the development of suitable imaging tracers [146]. Acetate is a metabolite known to accumulate in astrocytes through the activity of the monocarboxylate transporter (MCT). Since astroglial MCT expression is upregulated in MS lesions [126], astroglial accumulation of acetate is considered a feature of astrogliosis that can be measured by PET [199]. On this basis, ^{11}C-acetate has been proposed as a tracer. ^{11}C-acetate uptake significantly increases in the white and gray matter of MS patients, more predominantly in the white matter [178]. However, its overall low brain uptake renders ^{11}C-acetate inadequate for quantitative assessments [200]. The derivatives ^{18}F-fluoroacetate and benzyl ^{11}C-acetate have been proposed as alternatives [131, 145]. However, more work needs to be done to develop the current tracers to a suitable standard, as well as to identify new ones before PET imaging of astroglial activation can become a reliable tool.

An alternate approach to MRI that can overcome some of its limitations proving useful in MS is MR spectroscopy (MRS) [15]. MRS allows to monitor pathological processes via changes in their characteristic metabolic markers [39]. These processes include astroglial activation, that can be indirectly assessed by measuring myo-inositol (Ins) content [30]. Because Ins plays an important role in the control of astrocyte volume, elevated levels of Ins are considered an indicator of astrogliosis, thus of neuroinflammation. The ratios between Ins and other metabolic markers such as *N*-acetylaspartate (tNAA), a surrogate for axonal integrity, or creatine (tCr), a surrogate for cell proliferation, are used to define astrocyte reactivity and disease evolution in MS. Increased Ins levels can be observed in the normal appearing white matter compared to control white matter [39, 83], indicating that alterations associated with astroglial reactivity may be assessed early in disease and may play a role in its development. Elevated Ins/tNAA in the normal appearing white matter is a reliable predictor of brain atrophy, thus of disease progression and neurological disability evolution [99, 130]. Elevation of Ins/tCr has been shown to correlate with the disruption of neuronal networks, thereby serving as a marker and predictor of cognitive dysfunction in MS [172]. Also, an increase in Ins, Ins/tCr and Ins/tNAA in chronic inactive white matter lesions, independently from the disease course, has been detected, indicating ongoing astrogliosis even during the late stages of disease [130].

In parallel to imaging modalities, quantification of GFAP levels in serum and CSF have been proposed as options for assessing astroglial activation in MS patients. The presence of elevated GFAP levels in the CSF of MS patients compared to healthy individuals has been described by several groups. GFAP levels are associated with disease severity [151], with the highest concentrations found in secondary progressive patients, particularly those exhibiting severe ambulatory deficits [107, 140]. In general, GFAP is considered a prognostic marker of disability progression [16, 107, 110, 151]. In patients with early RRMS and clinically isolated syndrome (CIS), GFAP levels correlate with the intensity of gadolinium-enhancement on MRI scans, indicating that GFAP may be a biomarker of highly active disease in these populations [80]. More recently, levels of GFAP in the serum have been proposed as disease biomarkers. Serum GFAP levels are elevated in all disease types [2, 73], and highest with increasing MRI-lesion count, especially in progressive patients [2]. This suggests that serum GFAP could be a suitable disease progression marker.

A few studies have looked at S100β, another astrocytic protein considered a marker of astrogliosis, in correlation with disease severity and progression. Levels of S100β are higher in the CSF of MS patients, especially in RRMS [18, 140], and seem to distinguish them from SPMS and PPMS patients [140]. Nevertheless, more evidence is needed to validate S100β as a disease biomarker.

Collectively, these studies show that, weather measured by imaging techniques or biological testing of CSF and serum samples, in vivo assessment of astroglial reactivity in MS patients is a reliable early diagnostic and prognostic tool for MS. The routine implementation of these assessments

may in the future become the standard of practice, as it could be invaluable in guiding therapeutic decisions. More data need to be acquired in patients after treatment with disease-modifying drugs to determine whether these in vivo assessments of astrocytic activation may also be indicative of therapeutic efficacy.

Concluding remarks

The contribution of astrocytes to MS and EAE immunopathology is now established. In both, astrocytes undergo a process of cellular activation that is evident from the initial stages of disease. This results in astrocytes gaining the ability of producing a host of soluble mediators, neurotoxic and neuroprotective, as well as losing the ability of maintaining homeostatic functions important for CNS integrity. In their neurotoxic capacity, astrocytes produce oxidants, cytokines and chemokines that exacerbate immune activation and recruitment, thus causing tissue damage. In their neuroprotective capacity, astrocyte produce anti-inflammatory and pro-remyelinating factors, and contain indiscriminate CNS immune cell invasion through the formation of a glial scar. Despite this dichotomy, numerous studies in both MS and EAE have established a positive correlation between astroglial activation and disease severity and evolution, suggesting that strategies aimed at an overall suppression of astroglial activation may prove beneficial. Non-invasive measurements of astroglial activation in patients may represent a way to monitor disease severity, predict disease progression, and possibly test the efficacy of MS therapeutics. Overall, continued efforts to better understand the nature of astroglial responses in neuroimmune disease are warranted, with the EAE model and current methodological and technical advances providing valuable tools to understand molecular signals and mechanisms that could be targeted for therapeutic purposes in MS.

Acknowledgements We are grateful to Geoffrey Taghon for his contribution to the graphic design of Fig. 4. R.B. was supported by NIH-NINDS (grant 1R01NS094522-01), the Italian Multiple Sclerosis Foundation (grant FISM 2015/R/7), the US National Multiple Sclerosis Society (grant NMSS PP-1804-30716), and The Miami Project To Cure Paralysis.

Compliance with ethical standards

Conflict of interest The author declares no conflicts of interest.

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