Review

Astrocytes in the tempest of multiple sclerosis

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A R T I C L E   I N F O

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A B S T R A C T

Astrocytes are the most abundant cell population within the CNS of mammals. Their glial role is perfectly performed in the healthy CNS as they support functions of neurons. The omnipresence of astrocytes throughout the white and grey matter and their intimate relation with blood vessels of the CNS, as well as numerous immunity-related actions that these cells are capable of, imply that astrocytes should have a prominent role in neuroinflammatory disorders, such as multiple sclerosis (MS). The role of astrocytes in MS is rather ambiguous, as they have the capacity to both stimulate and restrain neuroinflammation and tissue destruction. In this paper we present some of the proved and the proposed functions of astrocytes in neuroinflammation and discuss the effect of MS therapeutics on astrocytes.

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1. Support and much more

Astrocytes are glial cells of the CNS which provide optimal physical and metabolic environment for neuronal activities. A common characteristic of all astrocytes is their star-like shape to which they owe their name (ancient Greek, αστρον – star, κύτος – cell). The other specificity of astrocytes is that their cytoplasm contains intermediate filaments composed of a distinct protein, glial fibrillary acidic protein (GFAP) [1]. Thus, we could describe an astrocyte as a neuroglial star-shaped cell containing GFAP. Beyond this simple description, astrocytes are a diverse cell population, with distinct properties in different CNS regions and at different periods of CNS development [2]. For instance, rodent astrocytes have been classified into two groups on the basis of their morphology and location, i.e., highly ramified protoplasmic astrocytes of the grey matter, which ensheath synapses and are in contact with blood vessels and fibrous astrocytes of the white matter, which are in turn in contact with the nodes of Ranvier [3]. Still, this classification into two groups might not be adequate to appreciate the full extent of astrocyte diversity, especially in humans, as human neocortex harbors several anatomically defined subclasses of astrocytes not represented in rodents [4]. Moreover, human astrocytes are up to three fold larger and more ramified than their rodent counterparts. These facts allowed Oberheim et al. to propose that astrocytic complexity has permitted the increased functional competence of the adult human brain [4]. The variety of functions that astrocytes perform within the CNS, implies that these cells are involved in almost everything the CNS does [5]. Indeed, as previously stated by Zhang and Barres, astrocytes are crucial for potassium homeostasis, neurotransmitter uptake, synapse formation and function, regulation of blood–brain barrier (BBB), myelination of axons and the development of the nervous system [2]. From neuroscientist’s point of view, maybe the most intriguing finding is that astrocytes intensively contribute to neurotransmission and regulation of sleeping, learning and memory [6,7]. Their importance for neurotransmission is highlighted in the concept of tripartite synapse, where astrocyte end-feet play equally important role for signal transduction as presynaptic and postsynaptic terminals [7]. There, in response to the increase of intracellular...
calcium concentration evoked by neurons, astrocytes release "glutamate, ATP and o-serine. It seems that new picture of brain function emerges in which slow-signaling astrocytes adjust fast synaptic transmission and neuronal firing to shape complex brain functions [6].

2. Gatekeepers and doormen

From the neuroimmunologist’s point of view, the contribution of astrocytes to BBB formation and function is among the most important roles of these glial cells, as BBB is the location where first interaction between CNS and immune cells happens in the process of neuroinflammation (Fig. 1). BBB represents one of the major contributors to CNS immune privilege and therefore studying its immune-related functions is of major importance for understanding pathogenesis of inflammatory demyelinating CNS disease multiple sclerosis (MS) and its various animal models, such as experimental autoimmune encephalomyelitis (EAE), Theiler’s murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD), herpes simplex virus (HSV)-induced encephalitis and murine hepatitis virus (MHV)-induced demyelination. The importance of astrocytes for BBB formation is clearly presented by their ability to induce tight junctions, high mitochondrial content and other intrinsic properties of neural endothelial cells, that build up the BBB, in endothelial cells of non-neural origin [8]. BBB-inducing actions of astrocytes are also prominent in heterologous system, where rat astrocytes and human umbilical endothelial cells or immortalized human endothelial cells are co-cultivated, thus implying that BBB-promoting factors made by astrocytes are not species-specific [9,10].

It is assumed that immune cells invade the CNS in two steps during neuroinflammation [11]. First, immune cells egress from postcapillary venules through endothelial cells and enter Virchow–Robin spaces (VRS). Subsequently, they interact with astrocytes of glia limitans and only if they subdue these cells, immune cells enter the CNS parenchyma (Fig. 1). Importantly, T cells have to cooperate with macrophages/microglia in order to penetrate through glia limitans into parenchyma [12], possibly due to specificities of glial basal lamina composition which makes it impermeable to lymphocytes, but permeable to macrophages [11]. Astrocytes are supposed to additionally limit this second step by induction of apoptosis in infiltrating cells. There is a constitutive expression of the death ligand CD95L on the astrocytic end-feet, and astrocyte-induced T cell apoptosis is dependent on CD95L [13,14]. This mechanism of T cell death could well be responsible for the perivascular apoptosis which is typical for established neuroinflammation, as seen in EAE [15]. Also, this mechanism could contribute to the prevention of intraparenchymal infiltration of T cells, and thus to the restrain of neuroinflammation. Still, astrocytes are also susceptible to CD95L-induced apoptosis and if large numbers of T cells enter VRS they could defeat astrocytes in their own game. For instance, it was shown that CD4+ T cells induce CD95L-mediated apoptosis in astrocytes in TMEV-IDD [16]. Notably, CD95L is not the only apoptosis-inducing molecular utilized by astrocytes, as these cells have shown capable of inducing apoptosis in rat and murine encephalitogenic T cells by the means of soluble factors, such as nitric oxide [17] and osteoectin and astrocyte-derived immune suppressor factor (AdIF) [18].

3. Masons and wreckers

Extracellular matrix (ECM) is becoming increasingly appreciated as an important component of neuroinflammatory cascade in multiple sclerosis. ECM is an extracellular part of all animal tissues, it provides structural support to the cells and regulates intercellular communication, as well as it is a potent source of inflammatory messengers [19]. Two different forms of ECM exist in animal tissues. The first one is the basal lamina – clearly defined deposition composed of networked glycoproteins and fibrous proteins, including collagen type IV, laminin, nidogen, and heparin

Fig. 1. Plethora of astrocytic actions related to neuroinflammation. Immune cells egress from postcapillary venules into Virchow–Robin spaces (VRS). There recently egressed macrophages (Mf), perivascular macrophages (PM) and microglia (Mg) affect glial basal lamina and pave the way for T lymphocytes into parenchyma. Within VRS and parenchyma astrocytes (A) influence invading cells in various ways. They induce apoptosis in T cells within VRS and in the parenchyma, produce MMP which affect intercellular extracellular matrix (ECM, dotted lines), generate osteopontin which potentiate Th1 and Th17 immune response. Matrix metalloproteinase (MMP)-degraded ECM components (black dots) have chemoattractive, neurotoxic and immunomodulatory effects. On the other hand, astrocytes produce tissue inhibitors of metalloproteinases (TIMP), which inhibit MMP function, thus contributing to tissue preservation. Astrocytes also present antigen to T cells, still their antigen presentation could be stimulatory to T cells if performed with appropriate co-stimulation or inhibitory to T cells if performed without proper co-stimulation. Cytokines produced by Mf, T and Mg from VRS and/or parenchyma stimulate antigen-presenting properties of astrocytes, production of chemotacticants (such as MCP-1), as well as generation of reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI). Astrocytic syncitin, product of human endogenous retrovirus, also stimulates ROI and RNI production. These molecules, as well as pro-inflammatory cytokines, affect neurons (N), oligodendrocytes (Od) and myelin sheet. Astrocytes also produce various neurotrophic factors (NTF) which support regeneration of neurons. They also provide essential factors for oligodendrocyte progenitor cells (OPC)-mediated regeneration of myelin.
sulfate proteoglycan. The second form of ECM is the interstitial matrix-loose network mostly composed of collagen I, and dependently of tissue type, variable amounts of collagen II and V, as well as different glycoproteins, such as fibronectin, vitronectin, tenasin and proteoglycans. Besides these basic constituents, ECM contains numerous other components, as well. Moreover, the elements of ECM exist in several different isoforms which allow for a possibility of tissue-specific biochemical diversification of ECM. Notably, biochemical variety is closely related to functional diversification of ECM [19].

Structural specificity of ECM is highly emphasized in the CNS parenchymal basal lamina made by astrocytes, which contains laminins 1 and 2, unlike endothelial basal lamina which express laminins 8 and 10 [20]. Significantly, extravasation into VRS is achieved through interaction of immune cells with laminin 8 in endothelial basal lamina, while neither laminin 1 nor laminin 2 is involved in the passage of the cells through glia limitans into CNS parenchyma [20]. Further, specificity of the CNS ECM is that its interstitial component contains small amount of rigid fibrous proteins, such as collagens, laminins, fibronectins and high amount of hyaluronan, chondroitin sulfate and heparan sulfate [21]. All of the components of the CNS ECM are synthesized by CNS resident cells, including astrocytes [22,23].

Recent studies have shown a complex modification in the CNS ECM during the course of EAE and MS, including altered expression of both basal lamina and interstitial ECM proteins [23–25]. As an example, perivascular fibrosis, a characteristic feature of chronic MS lesions is composed of fibrilar collagen, dyglican and decorin. It is supposed to function as a physical and a biological barrier that limits neuroinflammation and the expansion of MS lesions [25]. Importantly, astrocytes might be the cells that produce major collagen components of the fibrosis in MS [25], as they were shown to express these molecules in vitro [26]. As already mentioned, astrocytes induce apoptosis in T cells through generation of an ECM component osteonectin [18]. Thus, in these ways, astrocytes might contribute to the limitation of neuroinflammation in the CNS.

Moreover, a splice variant of fibronectin CS-1 (CS – connecting segment) is detectable in astrocytes at the edge of lesions in MS [27]. CS-1 segment of fibronectin is important for MS pathology as it is a receptor for leukocyte integrin α4β1, the major adhesion molecule for the cells invading the CNS [28]. In the process of immune cells admission into the CNS several adhesive interactions occur, such as between leukocyte function-associated antigen (LFA)-1 (αLβ2) and intercellular adhesion molecules (ICAM), as well as between α4β1 integrin and vascular cell adhesion molecule (VCAM)-1 [29]. The importance of α4β1 integrin for the immune invasion of the CNS has been practically shown through the efficacy of natalizumab, a monoclonal blocking antibody of α4 integrin subunit, in MS therapy [30]. Additionally, it has been shown in EAE in mice that it is α4β1 integrin and not α4β7 integrin that is crucial for the entry of immune cells into the CNS [31,32]. What’s more, a classical α4β1 integrin receptor – VCAM-1 expressed on astrocytes seems to be essential for infiltration of the CNS parenchyma by encephalitogenic T cells and for induction of neurological deficits in murine EAE [33]. In support of the importance of astrocytes as a source of VCAM-1 in neuroinflammation, it has recently been shown that TMEV induces expression of this adhesion molecule in murine brain astrocytes [34]. Thus, through expression of VCAM-1 and fibronectin CS-1 astrocytes actively contribute to the infiltration of cells expressing α4β1 integrin into the CNS parenchyma. Related to α4β1 integrin, osteopontin is another component of ECM that has a major impact on neuroinflammation. Osteopontin is upregulated in EAE, TMEV-IDD and MS, in which cases it is located in perivascular cuffs [19,28,35]. It stimulates infiltrating T cells through α4β1 engagement and directs these cells towards pathogenic Th1 and Th17 phenotype. Also, osteopontin stimulates expression of IL-2 which is significant for T cell survival, simultaneously inhibiting their apoptosis [19,28]. Significantly, astrocytes might be an important source of osteopontin during neuroinflammation, as shown in a rat model of systemic lipopolysaccharide injection [36]. Therefore, through both generation of CS-1 fibronectin and osteopontin, astrocytes might contribute to the tissue destruction in MS.

Important factors in ECM remodeling during inflammation are metalloproteases (MMPs), endopeptidases that belong to the family of at least 20 different members. They are transcriptionally regulated by various factors, such as proinflammatory cytokines, growth factors or hormones, while their activity in tissues is regulated by tissue inhibitors of metalloproteinases (TIMPs). MMPs disrupt basal lamina of BBB and contribute to parenchymal damage in neuroinflammation [37]. Infiltration of immune cells through parenchymal basal lamina requires activity of MMP2 and MMP9, which cleave distroglycan receptors leading to destabilization of astrocyte end-feet anchorage to parenchymal membrane. Further, MMPs selectively hydrolyze some matrix molecules, resulting in formation of bioactive peptides which play role of chemokactrants or immunomodulators, thus affecting activity of immune cells [19]. Expression of MMP 2 and 9 has also been detected in astrocyte cultures in vitro, as well as in MS lesions in situ [38,39], thus implying that astrocytes could contribute to ECM decomposition in neuroinflammatory plaques. Still, the ability of astrocytes to produce TIMP-1 was reported in EAE and TMEV-IDD in mice [40,41] and it has been assumed that astrocytes intensively contribute to the CNS tissue repair through generation of TIMP-1 and consequent influence on ECM maintenance and remodeling [42].

Finally, components of ECM might be essential for pro-inflammatory functions of astrocytes induced by IL-1β. This cytokine is considered to be a major activator of astrocytic expression and generation of chemoattractant and adhesion molecules for leukocytes in neuroinflammation [43]. Parenchymal astrocytes are in close contact with interstitial ECM, which is mainly built of non-rigid structures, including hyaluronan, tenasin-C, and proteoglycans. However, after BBB disruption, astrocytes become exposed to more rigid ECM components released from perivascular ECM, such as fibronectin, laminin, and fibrillins. These ECM components are also produced within the CNS parenchyma in response to injury or inflammation. Significantly, interaction of astrocytes with these components in vitro seems to be essential for IL-1β-induced reactivity of astrocytes [44].

4. Enemies within and friends in need

Although BBB is impermeable to most immune cells, CNS is constantly patrolled by low numbers of activated T cells which cross the intact BBB [45,46]. It has been proposed that activated encephalitogenic T cells penetrate BBB non-specifically and recognize CNS antigens on the surface of local antigen presenting cells within the parenchyma [47]. Besides dendritic cells, macrophages and microglial cells, MHC class II-inducible astrocytes are candidates for the initial presentation of autoantigens to infiltrating T cells in the CNS parenchyma [45]. Astrocytes have been shown to process and present major neuroinflammation-related autoantigens, such as myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG) to encephalitogenic CD4+ T cells in EAE [48,49]. Expression of MHC class II molecules on astrocytes was assumed to be crucial for antigen presentation within the CNS and therefore for pathogenesis of TMEV-IDD in mice [50]. Importantly, astrocytes seem to be the major hosts for TMEV within the CNS, thus are responsible for persistence of TMEV in the CNS, which additionally promotes them as important antigen-presenters in this disease [51].
infection of astrocytes with HSV and close contact of infected astrocytes with T cells were detected in HSV-induced encephalitis [52,53], thus implying that astrocytes might be significant presenters of antigens in this animal model, as well. Further, astrocytes seem to be the major cellular reservoir of neurotropic MHV [54], and MHV was shown to induce both MHC class I and MHC class II molecules on astrocytes [55,56]. This relationship suggests that astrocytes are deeply involved in antigen presentation to T cells in MHV-induced demyelination of the CNS. Still, antigen-presenting capabilities of astrocytes are questionable, especially in vivo. All the important molecules for efficient antigen presentation to and activation of CD4+ T cells, including MHC class II molecules and co-stimulators, such as B7-1, B7-2 and CD40 have been shown to be present on astrocytes in some experimental settings, but absent in others (reviewed in details by Dong and Benveniste and Chastain et al.)[57,58]. Thus, there is also a possibility that astrocytes present antigens in the absence of adequate co-stimulation, shifting CD4+ T cells towards regulatory phenotypes or inducing apoptosis or anergy in T cells [47]. Accordingly, astrocytes have been shown capable of inducing regulatory anti-encephalitogenic cells, which were able to protect animals from EAE [59,60].

Expression of antigen-presenting molecules in astrocytes is heavily dependent on the stimulation by pro-inflammatory cytokines, such as IFN-γ, TNF and IL-1β [57]. Also, their antigen-presenting capacity seems to be dependent on IL-12 and/or IL-23 [61]. These, and other, inflammatory mediators can act on astrocytes at very early stages of neuroinflammation, even before parenchymal infiltration of T cells, as it has been demonstrated that breakdown of the solute barrier occurs earlier than the cellular barrier in EAE [62]. This early leakage of soluble products of intravascular inflammatory cells through BBB generates a highly toxic environment resulting in vigorous astroglial responses and myelin and axonal damage at the time when parenchymal T cells are rare [63]. Astrocytic reactivity was observed before inflammatory infiltrates in EAE lesions [64], as well as in areas of MS lesions that lack a significant inflammation [65]. Moreover, astrocytic responses coincided with the earliest manifestation of axonal damage in EAE [63]. Evidence also exists for an early role for astrocytes in other disorders with a neuroinflammatory pathogenic component, such as amyotrophic lateral sclerosis [66], glaucoma [67], and Parkinson’s disease [68]. Notably, human endogenous retrovirus (HERV)-W family has been frequently associated with neuroinflammation, and MS in particular [69]. The HERV-W envelope-encoded glycosylated protein, syncytin-1, has increased expression in astrocytes in MS CNS. Overexpression of syncytin-1 in astroglia induces endoplasmic reticulum (ER) stress chanpores, such as the old astrocyte-specifically induced substance (OASIS). This chaperone, in turn, enhances astrocytic expression of inducible nitric oxide synthase (iNOS), thus stimulating production of NO, which has been among the most prominent damage-inducing molecules in neuroinflammation [70]. Astrocytes and microglia/macrophages are the most important source of iNOS in neuroinflammation and they have been shown to produce NO in response to various stimuli, including microbial products and components, cytokines and neurotoxins [70,71]. The importance of NO generation by astrocytes was shown in MS [72–74], as well as in EAE [75,76], TMEV-IDD [77] and MHV-induced encephalitis [78]. Two major pathogenic T cell populations in EAE and MS are Th1 and Th17 cells, with IFN-γ and IL-17 as their respective signature cytokines [79]. Interestingly, while both rodent macrophages and astrocytes express iNOS in response to IFN-γ, only astrocytes respond by further elevation of NO production in response to simultaneous stimulation with IFN-γ and IL-17 [80]. Also, rat astrocytes are capable of stimulating both IFN-γ and IL-17 synthesis in T cells [81]. Furthermore, astrocytic iNOS has been demonstrated in MS lesions, and it is recognized as an important pathogenic feature of MS [82].

Astrocytes are well capable of producing various pro- and anti-inflammatory cytokines, such as IL-1, IL-6, IL-10, IL-12, IL-15, IL-23, IL-27, IL-33, IFN-α, IFN-β, TGF-β1, TNF and various chemokines, including CCL2 (MCP-1), CCL3, CCL4, CCL5, CXCL20, CCL5 (RANTES), CXCL8 (IL-8), CXCL10 (IP-10) and CXCL12 (SDF-1) [57,61,83,84]. Thus, astrocytes could be involved in the complexity of cytokine functions in MS pathogenesis [88]. Here, we emphasize capability of astrocytes to produce IL-12 family cytokines, i.e., IL-12, IL-23 and IL-27, as these cytokines are among crucial for the direction of Th cells towards encephalitogenic Th1 and Th17 phenotype [85,89,90]. Also, astrocyte-derived IL-15 has been shown essential for the activation of encephalitogenic CD8+ cells in MS [91]. Further, it is proposed that reactive hypertrophic astrocytes contribute to the evolution of MS lesions through production of various pro-inflammatory mediators which attract immune cells to the lesions and/or activate these cells within the CNS parenchyma [92]. More specifically, reactive astrocytes produce MCP-1 (CCL2), a chemokine which has a crucial role in the recruitment and activation of myelin-degrading macrophages [92]. Once monocytes invade CNS parenchyma, the ongoing inflammation affects astrocytes. In heavily infiltrated areas of CNS massive loss of connexin43 (Cx43) expression is evident in EAE [24]. The loss of Cx43 affects astrocyte connectivity, as networking of astrocytes through gap junctions is dependent on this molecule. Importantly, regions of interrupted astrocytic communication also show axonal dystrophy, demonstrated by the abnormally dephosphorylated heavy-chain neurofilament proteins [24]. Astrocytes in these Cx43-depleted lesions are strongly GFAP-positive, which is a prominent characteristic of reactive astrogliosis [93]. In reactive astrogliosis, astrocytes intensively proliferate and migrate to the lesions. Their reactivity within the lesions might be pro-inflammatory and devastating, as exemplified in the previous part of the text. Also, these cells make a scar that prevents neuro-regeneration in the affected area [94]. However, in EAE mice that had transgenically targeted ablation of proliferating astrocytes, there was no astrogliosis and glial scar formation, and this was associated with a pronounced and significant increase in macrophage, T lymphocyte and neutrophil entry into CNS parenchyma, and finally with more severe and rapidly fulminant clinical course of the disease [95]. These findings show that besides negative effects of astrogliosis and glial scar formation, reactive astrocytes-formed perivascular barriers restrict the influx of leukocytes into CNS parenchyma and protect CNS function during neuroinflammation. Also, through production of anti-inflammatory cytokines and growth factors astrocytes contribute to the limitation of neuroinflammation and the repair of neuronal tissue [84]. Finally, the maintenance of basic metabolic functions of astrocytes seems to be essential for prevention of tissue destruction in neuroinflammation. As an example, it was shown in an autoimmune inflammatory disease neuromyelitis optica (NMO), once considered a subtype of MS, that the auto-antibodies specific to the aquaporin-4 (AQP4) water channel can induce astrocyte injury which may lead to the accumulation of excitotoxic molecules and accordingly to damage of oligodendrocytes and neurons [96].

Demyelination is a major pathologic feature of MS, while inefficient remyelination might cause a long-lasting neurological deficits in patients [97]. Besides infiltrating cells, microglia and astrocytes contribute to demyelination through phagocytosis of myelin and generation of molecules toxic to oligodendrocytes. The ability of astrocytes to perform phagocytosis of myelin was recorded in acute MS lesions, where hypertrophic astrocytes were identified as cells capable of myelin degradation and internalization of myelin debris through clathrin-coated pits [98]. Regarding toxicity of astrocytic products, for instance synctin-1 expression in astrocytes leads to the induction of various reactive species, such as superoxide anion and peroxyxidrite, to which oligodendrocytes are particularly vulnerable because of the level of antioxidants.
might be lower in this cell type [99]. Still, astrocytes are also important for myelin formation and prevention of demyelination as demonstrated in Alexander's disease, a demyelinating disease that emerges as a consequence of mutations in GFAP. Although the precise disease mechanisms are unknown, it is appreciated that the structural integrity of astrocytes, which provide a link between oligodendrocytes and BBB, seems to be critical for myelin preservation and axonal support [100]. Interestingly, in the model of HSV-induced CNS demyelination loss of astrocytes preceded loss of myelin, thus supporting the idea of the importance of astrocytes for myelin integrity [101]. Moreover, astrocytes produce a number of growth factors and related molecules that promote oligodendrocytic generation of myelin, although some of them have an opposite effect [102]. Through these factors and by other means, astrocytes also affect oligodendrocyte progenitor cells (OPCs) capable of remyelinating axons in the adult brain. It is supposed that astrocytes have the potential to significantly influence the extent to which the inflammatory lesion environment is supportive or obstructive to OPC recruitment and differentiation, oligodendrocyte survival, and remyelination [103].

5. Astrocytes as drug targets

Having in mind the importance of astrocytes for pathogenesis of MS and other neuroinflammatory diseases, it is rational to direct therapeutic treatment towards potentiation of beneficial and reduction of detrimental actions of these cells. Although so far there has been no astrocyte-specific therapy designed for the treatment of MS, beneficial effect of some of approved and candidate drugs for the treatment of neuroinflammatory disorders could be related to modulation of astrocyte functions. There are currently four drugs that are approved worldwide as therapeutics for MS patients, i.e., IFN-β, copaxone, mitoxantrone and natalizumab. Fingolimod could be added to this list soon, as it has recently been approved by U.S. Food and Drug Administration as an oral treatment of patients with relapsing forms of MS (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm226755.htm). Also, synthetic glucocorticoids are still widely used for the treatment of relapses in MS patients. Beside these, numerous drugs, including mycophenolate mofetil, rapamycin, tacrolimus, leflunomide, azathioprine, cyclophosphamide and cladribine have been studied for the efficiency in MS patients [104–105].

Numerous in vitro studies suggest that IFN-β restrains various inflammatory actions of astrocytes, such as antigen presenting capacity [106], cytokine and iNOS expression [107] and MMP generation [39]. Further, elevated levels of a putative astrocytic paracrine neurotrophic factor S100B are also observed in MS patients treated with IFN-β [108]. Glatiramer acetate (GA, Copaxone, Copolymer 1) also elevates expression of neurotrophic factors and anti-inflammatory cytokines in the CNS of EAE mice [109]. Fingolimod (FTY720), the first oral drug approved as MS therapeutic, acts as a functional antagonist of sphingosine 1-phosphate (S1P) receptors and its primary targets are S1P1 receptors on lymphocytes [110]. Through reduction of S1P1 signaling in lymphocytes, FTY720 slows egress kinetics of pro-inflammatory Th17 cells from lymph nodes, decreasing infiltration of the CNS and consequent neuroinflammation. Still, beneficial effect of fingolimod in neuroinflammation seems to be also dependent on its direct influence on astrocytes [110,111]. Down-regulation of S1P1 signaling in astrocytes reduce reactive astroglialis and improve gap-junctional communication among these cells, which might contribute to structural restoration of the CNS parenchyma in MS patients [110]. Synthetic glucocorticoids have been shown to affect various inflammatory functions of astrocytes, such as cytokine synthesis [112], MMP generation [113] and GFAP-related astrocytosis [114].

Mycophenolate mofetil has been shown beneficial in a randomized, blinded, parallel-group, pilot trial in MS patients [115], and it was previously shown that its bioactive metabolite mycophenolic acid inhibits iNOS-mediated NO generation in primary rat astrocytes [116]. Rapamycin (sirolimus) has recently been shown to modulate EAE [117] and to inhibit reactive astrogliosis in a model of spinal cord injury in rats [118]. Tacrolimus (FK506, fujimycin) is efficient in relaxing/remitting EAE in mice [119], and is currently under investigation in a clinical trial in MS patients in Canada (ClinicalTrials.gov Identifier: NCT00298662). This drug was reported to inhibit IL-1β and TNF synthesis in rat astrocytes [120]. Methotrexate has been shown beneficial in progressive MS [121], and it was reported that the drug had an intensive effect on astrocyte biology [122]. Leflunomide was shown effective in EAE [123] and its active metabolite teriflunomide has been shown beneficial in MS clinical trial [124]. Teriflunomide also down-regulates iNOS expression and NO generation by rat astrocytes [125]. Effect of mitoxantrone and natalizumab that are approved for MS therapy, as well as of Azathioprine, Cyclophosphamide and Cladribine that have beneficial effects in the disease [126–128], to the best of our knowledge, have not been investigated in relation to astrocyte functions in neuroinflammation.

Interestingly, fluoxetine (prozac, sarafem), an antidepressant of the selective serotonin reuptake inhibitor (SSRI) class I, has been shown to reduce the development of focal inflammatory lesions in MS patients [129]. Importantly, this drug activates protein kinase A (PKA) in astrocytes, which might be essential for prevention of MHC class II-dependent antigen presentation by these cells [130]. It has been previously reported that astrocytes in the white matter of subjects with MS are deficient in β2 adrenergic receptors [β2AR] [113]. Signaling through β2AR increases cAMP, leading to activation of PKA and subsequent phosphorylation and inactivation of coactivator class II transactivator (CIITA), which is a key regulator of MHC class II molecule transcription. Thus, β2AR deficiency reduces the suppressive action of PKA on antigen presenting capacity of astrocytes while fluoxetine might compensate this deficiency through inducing PKA in astrocytes [130]. Moreover, fluoxetine also stimulates secretion of neurotrophic S100B from astrocytes [131], the beneficial effects of the drug in MS, could be partially explained through enhanced neurotrophic potential of the affected CNS tissue. The effect of various drugs on astrocytes is presented in Table 1.

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<td>Glatiramer acetate</td>
<td>Elevation of neurotrophic factors</td>
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<tr>
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Having in mind the importance of astrocytes for pathogenesis of MS and other neuroinflammatory diseases, it is rational to direct therapeutic treatment towards potentiation of beneficial and reduction of detrimental actions of these cells. Although so far there has been no astrocyte-specific therapy designed for the treatment of MS, beneficial effect of some of approved and candidate drugs for the treatment of neuroinflammatory disorders could be related to modulation of astrocyte functions. There are currently four drugs that are approved worldwide as therapeutics for MS patients: IFN-β, copaxone, mitoxantrone and natalizumab. Fingolimod (FTY720), the first oral drug approved as MS therapeutic, acts as a functional antagonist of sphingosine 1-phosphate (S1P) receptors and its primary targets are S1P1 receptors on lymphocytes. Through reduction of S1P1 signaling in lymphocytes, FTY720 slows egress kinetics of pro-inflammatory Th17 cells from lymph nodes, decreasing infiltration of the CNS and consequent neuroinflammation. Still, beneficial effect of fingolimod in neuroinflammation seems to be also dependent on its direct influence on astrocytes. Down-regulation of S1P1 signaling in astrocytes reduce reactive astroglialis and improve gap-junctional communication among these cells, which might contribute to structural restoration of the CNS parenchyma in MS patients. Synthetic glucocorticoids have been shown to affect various inflammatory functions of astrocytes, such as cytokine synthesis, MMP generation and GFAP-related astrocytosis. Mycophenolate mofetil has been shown beneficial in a randomized, blinded, parallel-group, pilot trial in MS patients, and it was previously shown that its bioactive metabolite mycophenolic acid inhibits iNOS-mediated NO generation in primary rat astrocytes. Rapamycin (sirolimus) has recently been shown to modulate EAE and to inhibit reactive astrogliosis in a model of spinal cord injury in rats. Tacrolimus (FK506, fujimycin) is efficient in relaxing/remitting EAE in mice, and is currently under investigation in a clinical trial in MS patients in Canada. Methotrexate has been shown beneficial in progressive MS, and it was reported that the drug had an intensive effect on astrocyte biology. Leflunomide was shown effective in EAE and its active metabolite teriflunomide has been shown beneficial in MS clinical trial. Teriflunomide also down-regulates iNOS expression and NO generation by rat astrocytes. Effect of mitoxantrone and natalizumab that are approved for MS therapy, as well as of Azathioprine, Cyclophosphamide and Cladribine that have beneficial effects in the disease, to the best of our knowledge, have not been investigated in relation to astrocyte functions in neuroinflammation. Interestingly, fluoxetine (prozac, sarafem), an antidepressant of the selective serotonin reuptake inhibitor (SSRI) class I, has been shown to reduce the development of focal inflammatory lesions in MS patients. Importantly, this drug activates protein kinase A (PKA) in astrocytes, which might be essential for prevention of MHC class II-dependent antigen presentation by these cells. It has been previously reported that astrocytes in the white matter of subjects with MS are deficient in β2 adrenergic receptors [β2AR]. Signaling through β2AR increases cAMP, leading to activation of PKA and subsequent phosphorylation and inactivation of coactivator class II transactivator (CIITA), which is a key regulator of MHC class II molecule transcription. Thus, β2AR deficiency reduces the suppressive action of PKA on antigen presenting capacity of astrocytes while fluoxetine might compensate this deficiency through inducing PKA in astrocytes. Moreover, fluoxetine also stimulates secretion of neurotrophic S100B from astrocytes, the beneficial effects of the drug in MS, could be partially explained through enhanced neurotrophic potential of the affected CNS tissue. The effect of various drugs on astrocytes is presented in Table 1.
6. Final remarks

All of the findings presented here imply that astrocytes are among the crucial players in neuroinflammation. In some phases of neuroinflammatory diseases it might be beneficial to reduce astrocytic activity, yet in other loss or disruption of astrocyte functions may underlie or exacerbate the inflammation and pathologies associated with autoimmune diseases of the CNS, including MS. Therefore, a potential therapeutic approach for MS patients has to be carefully tailored and to include aspect of a drug influence on astrocytes. Future investigation should aim to elucidate if astrocytes are indeed as important for neuroinflammatory etiopathogenesis as suggested in many papers, including this one.

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References


