ABSTRACT. A reactive astrogliosis is a prominent feature of the response to injury in the central nervous system (CNS), and studies in both man and animals have provided a well-defined sequence of morphologic changes that accompany these events. However, the contribution of a reactive astrogliosis to disease pathogenesis has until recently, remained poorly understood. Now, with the development of transgenic mouse models of CNS injury and inflammatory demyelinating diseases such as experimental autoimmune inflammation (EAE), it has become possible to dissect out specific roles for astrocytes in the inflammatory events. The data strongly support multiple roles for the astrocyte in the evolution of the lesion that depends upon lesion stage and lesion topography. This brief review will highlight data that has been collected over the past decade from both patients and animal models that has provided a greater understanding of the complexities of the astrocyte response, and the role that these cells might play in lesion formation with particular emphasis on multiple sclerosis.

Key words: astrocyte, multiple sclerosis, experimental autoimmune inflammation (EAE).

A reactive astrogliosis is a prominent feature of multiple sclerosis (MS) lesions and the pathologic characteristics of the astrocytic response have been extensively reviewed (see for example 1-3). Around early (acute) inflammatory lesions, astrocyte reactivity is widespread. There is a gradient of reactivity from modestly swollen process-bearing cells in normal adjacent white matter (NAWM) to grossly enlarged globoid (hypertrophic) astrocytes suspended in an edematous parenchyma in the lesion center (see Figures 1 and 2). The lesion edge is frequently defined by a wall of astrocytes whereas in the lesion center astrocytes display diffuse immunoreactivity for the astrocyte-specific intermediate filament, glial fibrillary acidic protein (GFAP), and contribute to the fleshy edematous state of the tissue. In some acute MS lesions, astrocytic damage is extensive. In such cases, the perivascular layer of astrocyte processes forming the glia limitans is disrupted leaving an intact basal lamina with scattered astroglial endfeet still attached. Individual astrocyte processes are swollen and contain a mixture of polymerized and depolymerized filaments. As the lesion ages, hypertrophic astrocytes persist but begin to accumulate GFAP, and develop processes packed with dense bundles of glial filaments. GFAP immunoreactivity is intense, tissue edema decreases, and collectively, these cells provide a scaffold for surviving axons and other cellular elements within the chronic demyelinated lesion. These lesions are mostly devoid of oligodendrocytes and inflammatory cells are much reduced in number. Relapsing disease activity is accompanied by recurrent-inflammatory activity and a fresh wave of astroglial reactivity and hypertrophy particularly at the lesion edge (chronic active lesions). In chronic lesions it is not uncommon to observe astrocytes containing centrioles, indicative of recent mitotic activity, and a survey of human biopsy material demonstrated immunoreactivity for a mitotic marker in as many...
A reactive astrogliosis in animal models

The pattern of the astrogliotic changes found in MS lesions has many features in common with the response to other forms of injury in the CNS. For example, recent studies using a spinal cord injury model in the mouse have shown that the nature of the astrogliotic response is a function of both time and distance from the lesion center. Of particular interest are the observations that morphologically and phenotypically distinct populations of astrocytes can be identified. These include a population of newly proliferated astrocytes with elongated overlapping processes that form at the border of the lesion, which can be shown to contain the lesion and prevent the spread of inflammatory cells and neuronal loss into the adjacent tissue. Lineage-tracing experiments have indicated that newly generated astrocytes derive from populations of cells with progenitor characteristics.
Astrocyte contribution to lesion development

Using EAE in the mouse as an animal model for MS, studies have shown that activation of astrocytes, as determined by changes in the expression of GFAP, is an early event in lesion development, precedes clinical disease by several days, predicts severity of disease, and supports an important role for astrocytes in the initial events in EAE\(^9\)\(^-\)\(^10\). The initial events in lesion development and in clinical expression of disease in both MS and EAE involve loss of blood-brain barrier function (BBB). It is well recognized that factors produced by astrocytes are required for establishment and maintenance of changes in endothelial cells that form the BBB, and so studies have examined changes in astrocytes that could account for loss of barrier function in MS and EAE. Argaw and colleagues have studied the effect of cytokines known to induce an activated phenotype in astrocytes\(^11\)\(^-\)\(^12\). Using the macrophage-derived cytokine IL-1, they showed that activation of astrocytes with IL-1 led to induction of the transcription factor hypoxia-inducible factor 1 (HIF-1) and its target vascular endothelial growth factor A (VEGF-A) in astrocytes. They showed further that production of VEGF-A by astrocytes acts on endothelial cells via its receptor VEGFR2/flk-1 resulting in down-regulation or loss of the endothelial tight junction proteins claudin-5 (CLN5) and occludin (OCLN), culminating in focal loss of BBB function in lesioned tissue. Reconstitution with recombinant CLN5, but not OCLN, restored endothelial barrier properties and rescued the BBB permeability phenotype. Inactivation of astrocytic VEGF-A expression reduced BBB breakdown, decreased lymphocyte infiltration and tissue damage. Knockdown studies identified eNOS as the principal mechanism underlying the effects of VEGF-A on the BBB and systemic administration of the selective eNOS inhibitor cavastrin in mice abrogated VEGF-A-induced BBB disruption and pathology and protected against neurologic deficit in EAE\(^13\).

A role for astrocyte secretion of sonic hedgehog (Shh) has also been implicated in BBB regulation by Alvarez and colleagues\(^14\). They found that both the paracellular permeability and the intercellular transport activity of human BBB endothelial cells decreased in the presence of Shh or a PTCH1 agonist. They showed further that Hedgehog signalling induced the expression of tight junction proteins occludin and claudin5 in endothelial cells and down-regulated the chemotraction of leukocytes. Of particular interest to MS and EAE, they also showed that the proinflammatory cytokines interferon-gamma and tumor necrosis factor upregulated Shh in astrocytes in culture. Immunohistochemical studies of MS tissue showed that in control brain tissue and in normal-appearing white matter (NAWM) adjacent to MS lesions, astrocyte endfeet surrounding parenchymal vessels displayed Shh immunoreactivity, and BBB ECs expressed Smo and Ptc-1. Shh immunoreactivity was strikingly enhanced in hypertrophic astrocytes throughout active demyelinating MS lesions. In NAWM, Ptc-1 expression was mainly restricted to ECs surrounded by astrocytic endfeet, whereas in active MS lesions, Ptc-1 expression increased on ECs and was also detected on infiltrating leukocytes. They suggest that,“ inflammation activates Shh production in astrocytes in order to promote BBB repair and counterbalance inflammatory events induced during lesion formation,
and thus restores physiological and immunological BBB competence\(^\text{15}\). Following ischemic insults, Shh has also been found to trigger Angiotensin-1 production in astrocytes, which upregulates ZO-1 and occludin in brain endothelial cells to restore tight junction activity and to ameliorate brain edema and BBB leakage\(^\text{15}\).

**Inflammation and the glia limitans**

In addition to tight junctions on endothelial cells, astrocytic endfeet that form the glial limitans provide an additional barrier to the entry of cells and serum proteins into the CNS. Quantitative analyses estimate that astrocytic endfeet cover anywhere from 80-100% of the endothelial basal lamina, and that microglial processes cover up to 13% of the vascular wall\(^\text{16}\). In capillaries in the CA1 stratum moleculare of the hippocampus of 7 week old rats the basal lamina underlying endothelial cells is completely (100%) covered by astrocytic endfoot processes\(^\text{17}\). However, the primary site of extravasation of inflammatory cells into the CNS parenchyma during EAE occurs at the level of the postcapillary venule\(^\text{18}\). In these vessels the single basal lamina of brain capillaries, which is composed of laminins 8 and 10, splits and a second basal lamina called the parenchymal basement membrane (BM) or basal lamina, which is composed of laminins 1 and 2, is produced by astrocytes and leptomeningeal cells. Astrocytic endfeet are attached to this parenchymal BM via dystroglycan interactions with the endothelial isoform of the heparin sulfate proteoglycan, agrin\(^\text{19}\). Encephalitogenic T cells breach these barriers in a step-wise fashion\(^\text{20}\). Activated T cells can effectively cross the endothelial cell and its associated basal lamina however metalloproteases (MMPs) secreted by macrophages are required for T cell migration across the parenchymal (astrocytic) barrier. Each step of this double-barrier migration process involves distinct molecular mechanisms, with the second step being the disease-relevant step\(^\text{17, 21}\). The reversible selective gelatinase activity of MMP2 and 9 for beta-dystroglycan is also selectively involved since no general digestion of parenchymal BM components was detected\(^\text{19, 22}\). Expression of MMPs 7 and 9 are upregulated in MS lesions\(^\text{23}\), and broad inhibition of MMP activity has been found to reduce immune cell trafficking into the CNS and attenuate EAE\(^\text{23-26}\). Tissue inhibitors of metalloproteases (TIMPs) are endogenous inhibitors of MMPs and are found in astrocytes in active demyelinating lesions in EAE\(^\text{27}\). Inactivation of TIMP-1 in mice led to enhanced inflammation in the CNS parenchyma, accompanied by increased microglia/macrophage activity and prolonged myelina/macrophage activity and prolonged myelin injury following sensitization for EAE\(^\text{28}\).

The choroid plexus blood-CSF barrier is also an early and essential site of entry of T cells into the CNS in EAE\(^\text{29}\), and entrapment of CD45 positive cells between the basement membrane of the choroid plexus epithelial cells and the laminin-positive endothelial basal lamina of the capillaries and venules inside the choroid plexus has also been noted. Transmigration of cells at this site this site is regulated by the chemokine CCL20 and adenosine, signaling through the A\(_2\) receptor\(^\text{30}\). In tissues from patients with MS, high CCL20 expression is detected in inflamed areas in astrocytes positive for GFAP as well as within the choroid plexus\(^\text{29}\).

**Astrocytes, Cytokines and Chemokines**

It is now well recognized that astrocytes can be activated in vitro to produce inflammatory cytokines and chemokines that can recruit lymphocytes to the CNS (reviewed in\(^\text{31-32}\)), and in vivo studies have confirmed that astrocytes can be induced to express biologically active cytokines in a dose- and time-dependent fashion. Furthermore, these astrocyte-derived cytokines activate appropriate signaling pathways which may be distinct from expression in other cell types, for example neurons\(^\text{33}\). To determine whether astrocyte cytokine interactions modulate inflammation in the CNS, Bethea and colleagues induced EAE in transgenic mice with a dominant negative NF-kB superrepressor under control of the GFAP promoter\(^\text{34}\). Remarkably, these mice showed significantly less severe clinical signs of disease and reduced evidence of demyelination and neuronal loss compared to WT mice, particularly during the chronic disease phase. Transgenic animals displayed significantly decreased levels of both cytokines and chemokines during all phases of the disease, indicating that signaling pathways in astrocytes are activated in EAE. However, TNFα immunoreactivity was noted only in microglia and macrophages, not astrocytes, thus reduced levels of this cytokine in the lesion likely reflects cross-talk between astrocytes and microglia/macrophages. Unexpectedly, mice expressing the astrocyte-directed NF-kB super-repressor actually exhibited increased leukocyte infiltration, which the investigators attributed to greater numbers of protective, CD8+/CD122+ regulatory T cells. These data indicate that astrocytic activation of the NF-kB pathway regulates TNFα, IFNγ, and chemokine expression in the CNS, thus both directly and indirectly regulating the severity and inflammatory cell profile of the lesion\(^\text{34}\). Astrocytes associated with the BBB have also
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...been reported to be the first cells in the CNS to be activated by MOG-reactive Th17 cells to make pro-inflammatory cytokines and chemokines essential for the induction of EAE. Interestingly, the pattern of cytokines induced in astrocytes by IL-17 plus TNFα (CXCL1, CXCL2, CCL20) differed from those activated in astrocytes by the Th1 cytokine IFNγ plus TNFα (CXCL9, CXCL10, CXCL11) demonstrating that chemokines secreted by astrocytes may regulate the nature of T cells and other leukocytes that infiltrate the CNS.

The contribution of activated astrocytes to lesion development and repair has also been tested in mice in which deletion of STAT3 has been targeted to astrocytes using a mouse GFAP-Cre-loxP system creating a conditional knock-out (CKO). STAT3 is activated by a number of cytokines implicated in the injury response several of which including IL-6, CNTF, LIF, EGF, and TGFβα have been implicated as triggers of a reactive astrogliosis. Thus, these studies examined the role of astrocytes as targets of the inflammatory cascade. Following spinal cord injury in these mice, the data showed that astrocyte hypertrophy and upregulation of GFAP were attenuated in STAT3 astrocyte CKO mice. Lesions in these animals showed a pronounced disruption of glial scar formation and displayed increased extravasation of CD45 positive cells into the peri-lesioned area, increased myelin loss, increased volume of necrotic tissue, and reduced functional recovery. Studies in vitro supported an important role for regulation of reactive oxygen species and ATP production in this process. Thus, these studies identified a beneficial role for activated astrocytes and development of the astrocytic scar in lesion resolution, and complement the diversity of responses noted following astrocyte-targeted expression of cytokines such as IL-6, TGFβ, TNFα, and IL-12 and chemokines.

Astrocytes act as a source of cytotoxic factors

Activated astrocytes also secrete compounds with potentially direct toxic effects on neurons/axons and oligodendrocytes/myelin such as reactive oxygen and nitrogen species, glutamate and ATP. In rodents, treatment with IFNγ or LPS causes a subset of astrocytes to produce inducible nitric oxide synthase (iNOS, also known as NOS-2). Combination treatment with TGFβ plus IFNγ increased the percentage of astrocytes secreting NO. Human astrocytes do not respond to LPS, but upregulate iNOS following activation with IL-1beta plus IFNγ or the toll-like receptor 3 (TLR3) ligand polyI:C. In situ hybridization and immunohistochemistry showed extensive iNOS reactivity in hypertrophic astrocytes in acute but not chronic MS lesions. Nitrotyrosine, a footprint for peroxynitrite, was also detected in parenchymal and perivascular regions in these acute lesions. Decreased uptake of glutamate by astrocytic and/or oligodendrocytic glutamate transporters could also contribute to pathologically elevated levels of extracellular glutamate, which is also directly toxic to oligodendrocytes and neurons. Glutamate excitotoxicity is thought to contribute to oligodendrocyte and axonal loss in MS, and glutamate receptor antagonists ameliorate EAE. Oligodendrocytes in human, rodent and rabbit optic nerve also express the ATP-sensitive purinergic receptor P2X7, and excessive P2X7 activation has been implicated in oligodendroglial cell death and axonal injury in EAE and MS.

Astroglial purinergic signaling

Astrocytes are electrically nonexcitable cells. Signalling through release of ATP and activation of P2 purinoceptors is the most widespread means of communication between astrocytes and other types of neural cells (reviewed in ). Astrocytes express various types of functional metabotropic P2Y purinoceptors and ionotropic P2X purinoceptors which play an important role in cell-cell communication via transient elevations of cytosolic Ca++. There are seven subtypes of ionotropic (cationic) P2XRs and eight metabotropic G-protein-coupled P2YRs, each with different ligand preferences (P2Y1: ATP; P2Y2: ADP; P2Y6: UTP; P2Y7: UTP; P2Y11: ATP; P2Y12: ADP; P2Y13: ADP and P2Y14: UDP-glucose and other nucleotide sugars). Studies have shown that cytokines such as IL-1 can modulate the expression of purinoceptors on astrocytes, thus providing a mechanism whereby astrocytes can both sense and respond to changes in the extracellular environment, perhaps also contributing to the role of astrocytes as sensors of ‘danger signals’. This may have important consequences at sites of injury since, for example, stimulation of astroglial P2YRs is involved in the secretion of glutamate, the regulation of several cytokines/chemokines, and expression of the cytotoxic P2X7 receptor.

Astrocytes role in lesion repair

Historically, the glial scar has been considered to have a negative impact on recovery from injury due to the classic role of the glial scar in inhibiting axonal regrowth (reviewed in ). Despite the clear inhibitory role for the glial scar in attenuating axonal regrowth and remyelination, the dense network of...
the glial scar comprises a diffusion barrier that protects the surrounding tissue from secondary degeneration caused by elevated levels of extracellular potassium, glutamate, and purines. Extracellular matrix molecules also bind to and concentrate cytokines, chemokines, and growth factors that contribute to repair, as has been recently extensively reviewed.

**Astrocytes as targets of an immune response**

In EAE, antigens associated with myelin are the focus of the immune response and thus changes occurring in astrocytes are considered as ancillary to a T cell dependent attack on myelin. That antigens associated with the astrocyte may be the target of an immune response was first demonstrated in the rat, where it was found that the astrocyte-derived calcium-binding protein S100β could induce inflammatory changes in the CNS. More recently, however, it has now been well established in patients with neuromyelitis optica (NMO, also known as Devic’s disease) that antibodies specific for the water channel aquaporin 4 (AQP4) in the circulation constitute a biomarker for the disease. AQP4 is a water channel that in the CNS is predominantly localized to astrocytic foot processes that abut the glia limitans at the blood-brain barrier (BBB). At this site AQP4 forms a macromolecular complex with the glutamate transporters GLAST, GLT1 and the inwardly rectifying potassium channel Kir4.1, which is anchored to the glial basal lamina via interactions with alpha-syntrophin. However, following injury, this polarized expression of AQP4 is lost and immunoreactivity for AQP4 is distributed along the cell processes.

Sites at which AQP4 immunoreactivity is lost in NMO also show evidence of immunoglobulin deposition and complement activation. However, no similar antibodies or complement deposition have been found in MS patients, indicating that MS and NMO spectrum disorders are distinctly different, both serologically and histopathologically, and do not support a role for an antibody-dependent immune response in mediating the damage to astrocytes in the acute MS lesion. Nevertheless, in our own studies, early damage to perivascular astrocyte endfeet and to hypertrophic astrocytes in the adjacent parenchyma in acute MS lesions was unambiguous, but whether this was a primary event was difficult to evaluate due to concomitant edema and inflammation in these acute lesions. Taken together with findings documented in the literature from both the human disease and animal models, the data strongly support multiple roles for the astrocyte in the evolution of changes encountered in MS that depends upon lesion stage and lesion topography.

**Concluding remarks**

The availability of molecular and immunological tools that permit targeting of specific mediators to astrocytes in mice has provided a wealth of data that has shed new light on the role of astrocytes in the inflammation in the CNS. As can readily be appreciated from the present and other recent reviews, new techniques have illustrated just how complex this response is likely to be. However, it is important to point out that astrocytes in the human brain have many features that differ in several ways from that found in animals. Aspects of the pathological changes noted in MS lesions, such as multinucleated astrocytes and astrocytic uptake of oligodendrocytes and lymphocytes, have not as yet been described in EAE. In the human brain, the ratio of astrocytes to neurons is greater than that found in any other species, and human astrocytes are also larger, structurally more complex, and more diverse than those in rodents. The relevance of these differences to CNS function has recently been strikingly documented in mice chimeric for human and mouse astrocytes. Although cells of human origin were found to integrate with mouse cells, they retained their larger more complex structure. Most dramatically, mice chimeric for human and mouse astrocytes showed enhanced learning, as assessed by Barnes maze navigation, enhanced object-location memory, and both enhanced contextual and tone fear conditioning, indicating that human glia differentially enhance both activity-dependent plasticity and learning in mice. These new models make for exciting times ahead as we continue to pursue the phenotypic and functional properties of cells of the astrocyte lineage.

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