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Pillars of Immunology

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Glial Cells as Regulators of Neuroimmune Interactions in the Central Nervous System

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Neuroimmune interactions contribute both to physiologic and pathologic conditions within the CNS. The former includes brain development and learning, host defense against pathogens and tumors, and contributions to tissue repair following injury (1–3). Although regarded as a site of relative immune privilege based on persistence of brain allografts, the CNS has long been recognized as a potential target of autoimmune responses (4, 5). Multiple sclerosis (MS) has been the most commonly considered human disorder within the latter category. There is now, however, increasing recognition of specific Ab-linked disorders, including anti-aquaporin 4 Ab-associated neuromyelitis optica, myelin oligodendrocyte glycoprotein (MOG) Ab-mediated demyelinating disease, and NMDA-receptor–directed autoimmune encephalomyelitis (6–8). This *Pillars of Immunology* commentary will describe progress made in our understanding of the contribution of resident cells in the CNS to these dynamic processes. Our focus will be on cells within the CNS parenchyma, namely microglia and astrocytes, while recognizing the contribution of myeloid cells in "brain adjacent regions" that include the perivascular and meningeal spaces (9–12). We describe the evolution of this field from the initial work characterizing the properties of the microglia and astrocytes in isolation to the more complex challenge of defining the molecular pathways they use to interact with other cell types as well as with each other. The former includes components of the immune system that access this compartment and other neural cells, namely oligodendrocytes and neurons. Among glial cells, microglia and astrocytes serve as sensors of events occurring within the CNS, with response to these stimuli determining their phenotypic and functional properties. Analyses of such properties need to consider species, age, and regional variations (13–16) as well as sex-linked differences (17).

Characterization and role of myeloid cells in the CNS

Microglia were initially recognized as a constituent of the CNS parenchyma by classical histologic criteria. Lineage-tracing studies indicate that microglia are early derivatives of the yolk sac and populate the CNS prior to development of the systemic innate immune system (2, 3). Congenital absence of microglia links to significant deficits in brain development in humans (18). Complicating the analysis of microglia is the need to distinguish these cells from blood-borne myeloid cells that infiltrate the CNS parenchyma, especially under conditions with a disrupted blood–brain barrier (BBB) and the so-called border-associated macrophages, which reside in the perivascular and/or meningeal spaces (9–12). There is also a rare population of dendritic cells, dwelling primarily in the meningeal spaces and the choroid plexus, that present myelin Ags and are essential for T cells to initiate inflammation in the experimental autoimmune encephalomyelitis (EAE) model (11). To be further defined is the role of the various myeloid cells within the CNS parenchyma and in the meningeal spaces to persistent or chronic inflammation within the CNS, as has been implicated to underlie the progressive course of MS.

Our selected *Pillars of Immunology* paper (19) (https://www.jci.org/articles/view/118946) was based on analyses of adult human microglia that we were able to establish in dissociated cell culture because of our access to surgically resected noninflammatory, nontumor tissue samples (20). The availability of such cells contributed to continuing efforts to define the molecular signals underlying microglial responses to disturbed homeostasis in the CNS microenvironment and, in turn, how these microglia may contribute to immune responses. The percoll gradient–based isolation procedure that we use has been described in detail and is designed to remove contaminating myelin debris that inhibits in vitro cell survival (19, 20). Microglia isolated in this manner will survive in a postmitotic state in dissociated cell culture in an absence of growth factor support over a number of weeks, exceeding what is observed using mature rodent-derived samples (15, 16). Human fetal CNS-derived microglia do undergo a degree of proliferation in vitro. Viability of microglia, as well as oligodendrocytes, derived from such surgical samples exceeds that which is usually obtained when using autopsy-based material. In our study, we showed that stimulated adult human microglia produce IL-12p40 upon exposure to LPS, with the response being dependent on TNF production that acted in an autocrine loop (20). Such results implicated

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Abbreviations used in this article: BBB, blood–brain barrier; EAE, experimental autoimmune encephalomyelitis; MS, multiple sclerosis.

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microglia in locally providing a cytokine required for activation and maintenance of type I immunity. Subsequent studies established that the IL-12p40 subunit is shared by another cytokine, IL-23 (21). IL-23 and the ability of microglia to produce IL-23 is vital to maintain T cell encephalitogenicity within the CNS (22–24).

Our early investigations with human brain-derived microglia highlighted distinct features of these cells compared with macrophages (25). Human microglia showed a wider range of expression of TLRs compared with those derived from inbred "clean" animals (25, 26). Microglia are now shown to express a series of distinct molecules that regulate their response to signals from their microenvironment. These include TREM2 (27); mutations in this gene are linked to susceptibility to Alzheimer disease (27). In vitro and in situ microglia express an array of checkpoint inhibitory molecules, including PDL-1, CTLA-4, and TIM-3 (28, 29). Subsequently, we used nanostring technology to compare the molecular signature of adult human microglia maintained in dissociated cell culture with that of blood-derived macrophages under basal, proinflammatory, and TGF-β–supported conditions (30). These defined a TGF-β–dependent homeostatic signature that could then be used for comparison of such cells under neuroinflammatory and neurodegenerative conditions.

Microarray analysis indicated that our human adult microglia favored production of neurotrophic molecules compared with macrophages under similar polarization conditions, being most apparent in cells polarized into an M2 phenotype (31). This latter phenotype has been shown to link with the capacity of microglia to contribute to remyelination following toxin-induced demyelination (32).

We had observed that in vitro exposure of human microglia to supernatants from pro- and anti-inflammatory (Th1 versus Th2) T cells drives the cells toward distinct states of polarization (33). Bulk RNA-sequencing studies of freshly isolated microglia, confirmed by single-cell sequencing (13, 14, 34), indicate that these cells have a wide diversity of molecular phenotypes in situ, including regional heterogeneity, in line with the concept that they are responding to multiple signals. Such studies raise the issues of what the homeostatic state of the cells is for the outbred human population living in a dirty environment, and is there an expected state of polarization of these cells. Indeed, metabolites produced by the commensal flora regulate microglial activity under homeostatic and disease conditions (35–37).

A highly relevant function of myeloid cells in the CNS in context of demyelinating disease (MS) is tissue repair via the clearance of myelin debris. Myelin debris is inhibitory to migration and differentiation of progenitor cells that are required for the remyelination process. In vitro and in situ studies indicate that this process is dependent on specific molecular receptors, with Mertk, a member of the TAM family of receptors, being centrally involved (38). Our own studies have shown that human microglia are more efficient at Mertk-dependent myelin phagocytosis than are macrophages (38). Mertk receptor is more highly expressed by microglia compared with macrophages and is downregulated in myeloid cells under proinflammatory conditions (38). Such downregulation would be of potential benefit in the setting of myelin-driven autoimmune disease, as the proinflammatory myeloid cells with their upregulated MHC and costimulatory molecules would have reduced amounts of Ag to present (38). Mertk receptors are also involved with uptake of apoptotic T cells, favoring induction of an anti-inflammatory response (39). Pharmacologic inhibition of microglia using CSF-R1 blockers now provides a valuable approach to assess disease-mediating and protective functions of microglia during the course of experimental neurodegenerative diseases, such as models of amyotrophic lateral sclerosis and Alzheimer disease, particularly if, unlike the autoimmune disorders, systemic-derived macrophages are not considered to be significant contributors (15, 16).

Characterization and role of astrocytes in the CNS

The initial concepts of astrocyte function regarded these cells as mainly providing structural support to the CNS (40). They were also increasingly recognized as cells that provide metabolic support to neurons (40). Of note, aquaporin 4, which is expressed on astrocytes, represents the antigenic target in neuromyelitis optica (6). As with myeloid cells, defining the neuroimmunologic properties of astrocytes has evolved in concert with techniques to manipulate the cells in situ and to isolate the cells for in vitro analyses (41, 42). For the latter, most studies, especially as related to human cells, have been conducted using cells derived from the developing CNS, usually in the second trimester. Such cells have a significantly higher rate of proliferation compared with adult CNS-derived cells (39). Well recognized is that astrocyte scar formation is substantially different in the fetal and adult CNS (40). Although protocols for isolation of adult astrocyte cultures are described, no consensus method for deriving and maintaining such cells in enriched dissociated cell culture has been achieved (43, 44).

Contribution of astrocytes to the BBB

As reviewed in Refs. 45–49, the BBB regulates the exchange of cells and soluble molecules between the CNS and the periphery. The BBB is a complex structure formed by specialized capillary endothelial cells, an interstitial space containing immune-competent cells, including pericytes and blood-derived macrophages, and a second limiting membrane formed by astrocytes (glial limitans) and microglia processes (45, 50). The integrity of the tight junction barrier formed by endothelial cells is dependent on specific molecular signals, including the wnt-β catenin pathway, the Hedgehog pathway, TGF-β, brain-derived neurotrophic factor (BDNF), and angiotensin (49, 51–53). These molecules, secreted by perivascular astrocytic end-feet and, sometimes, by pericytes and microglia, also impact on additional BBB-related properties, including nutrient transporters and extrusion molecule expression (such as Pg-P) as well as downregulation of cytokine and chemokine receptors and adhesion molecules (46–49). Both astrocytes and microglia, as well as pericytes, are sources of molecules (chemokines, cytokines) that regulate the integrity of the barrier and properties of the cells contained within the perivascular spaces (46). Cytokines, secreted locally by infiltrated lymphocytes and myeloid cells, have the capacity to counter-act astrocyte signals and to destabilize the BBB, leading to breaches and expression of cell adhesion molecules, which further promote immune cell infiltration (51–53).

Immune regulatory/effector role

Both fetal and adult human astrocytes can be induced in vitro to express MHC class II molecules in response to proinflammatory

cytokines and TLR-directed signals, an effect that can be reproduced by in vivo administration of such agents (41, 42). Astrocytes are significantly less efficient than myeloid cells as APC, with limited expression of requisite costimulatory molecules (54). Astrocytes, however, can present superantigens in vitro (55). Their capacity to phagocytose is also significantly lower than myeloid cells, although astrocyte ingestion of whole oligodendrocytes and myelin debris have been described in MS and EAE lesions (56).

Astrocytes are sources of chemokines and cytokines that can shape the immune repertoire as well as mediate effector functions. Initial studies tended to focus on production of individual molecules. Examples of these include production of IL-15 and IL-27 that regulate the function of CD4 and CD8 T cells (57, 58). Touil et al. (59) demonstrated that secreted factors from human fetal astrocytes, both nonactivated and after exposure to proinflammatory cytokines, supported human B cell survival, although the effect could not be attributed to one specific cytokine. Liddelow and Barres (60) provided a transcriptome profile of astrocytes that showed neurotoxic activity following exposure to LPS. They found that neurotoxic astrocytes characterized by the expression of complement C3 and other molecules were detectable in various human neurodegenerative diseases, including Alzheimer, Huntington and Parkinson diseases, amyotrophic lateral sclerosis, and MS. The authors did point out that there was a heterogeneity of astrocytes, which was dependent on environmental stimuli. In this regard, conditioned media from human fetal astrocytes pre-exposed to Th1- but not Th2-polarized T cell–derived supernatants decreased the differentiation of human $A2B5^+$ neural progenitors oligodendrocyte progenitor cells (61). The effect was mediated through CXCL10. The regulation of astrocyte responses by T cell products is further illustrated by findings that IL-10 produced by type 1 regulatory T cells (Tr1 cells) attenuates astrocyte activities thought to contribute to the pathogenesis of MS (62). Interestingly, IL-27 promotes Tr1 cell differentiation (63–65). Collectively, these findings highlight bidirectional astrocyte–T cell interactions involved in the regulation of CNS inflammation.

The contribution of astrocytes to the course of neuroinflammatory disease has been explored in vivo by assessing the impact of astrocyte deletion during the acute and recurrent phases of EAE. The disease is worsened when reactive astrocytes are deleted during acute EAE, suggesting a contribution of these cells in limiting inflammation, perhaps by limiting the recruitment of peripheral inflammatory cells (66, 67). Disease severity is subsequently alleviated in chronic progressive EAE, suggesting a pathogenic contribution. A transcriptome analysis of astrocytes derived from EAE animals documents shifts in the transcriptional programs of astrocytes at multiple disease stages (66). Translating these observations to human MS provides a number of challenges and complexities. In MS, unlike the EAE model, the time of lesion formation cannot be precisely identified, and such new lesions may occur at or near sites of previous lesions and/or in normal-appearing white matter that has evidence of ongoing astrocyte and myeloid cell activation. Alvarez et al. (68) have described "pre-lesional" changes in the normal-appearing white matter of MS patients, with important signs of BBB breaches prior to immune cell infiltration and in the absence of demyelination. These are believed to be the first elements of lesion formation in human MS.

Our analysis of microRNA expression in astrocytes microdissected from the human brain (69) further emphasizes astrocyte heterogeneity under basal and disease conditions. The microRNAs analyzed were selected based on their demonstrated involvement with regulating structural properties of astrocytes and their involvement in ischemic and inflammatory responses (69). We showed differences between gray and white matter expression as a function of age (fetal versus adult) and between acute, chronic-active, and chronic MS lesions versus controls (69, 70). Of specific interest was expression of the proinflammatory regulator mir155, which was not upregulated in astrocytes in acute MS lesions (70). This finding contrasts to its upregulation in fetal human astrocytes exposed in vitro to IL-1β/IFN-γ and its upregulation in myeloid cells microdissected from acute inflammatory lesions (70, 71). These data emphasize that functional properties of astrocytes in the context of MS (and likely other pathologic processes) are driven by multiple competing stimuli. Astrocytes are simultaneously participating in both injury and repair processes under pathologic conditions, while also seeking to execute their physiological roles.

Specific pro- and anti-inflammatory regulatory networks have now been identified within astrocytes. Mayo et al. (66) found that the enzyme B4GALT6 is upregulated in astrocytes recovered from animals undergoing chronic progressive EAE. This led to the definition of a pathway dependent on sphingolipid metabolism involving lactosylceramide that drove a proinflammatory response in astrocytes. Knockdown of this pathway resulted in increased expression of anti-inflammatory (e.g., *Il-10*, *Il-27*, and *Socs2*) and neurotrophic factors (*BDNF*, also known to promote BBB integrity), with concomitant decreased expression of inhibitory factors of axonal growth (e.g., *Sema3a* and *Rgma*) (66). In vitro studies confirmed the activity of this pathway in human astrocytes (66). Targeting of sphingolipid signaling with FTY720 in vitro led to a decrease of proinflammatory chemokines (*Ccl2*), cytokines (*Csf2*, *Il-6*, *Tnfa*), and neurotoxic- (*Nos2*, *Tnfa*) and macrophage-polarizing factors (*Csf2*) (72). Rothhammer et al. (73) detected a transcriptional response to IFN-I in astrocytes during experimental CNS autoimmunity and also in CNS lesions from MS patients. IFN-I signaling in astrocytes limits inflammation-driven neurodegeneration through a mechanism mediated by the ligand-activated transcription factor aryl hydrocarbon receptor (AhR) and suppressor of cytokine signaling 2 (SOCS2). The AhR pathway is regulated by multiple small molecules, including microbial metabolites derived from dietary tryptophan (73). Thus, microbiome manipulation or the generation of synthetic mimics of microbial molecules may provide a therapeutic approach for the modulation of glial responses. More recently, Wheeler et al. (74) found that the herbicide Linuron induces disease-promoting activities in astrocytes by activating the inositol-requiring enzyme-1α (IRE1α)/X-box binding protein 1 (XBP1) via the sigma receptor 1 (Sigmar1), further linking environmental factors with the activation of specific signaling pathways in astrocytes.

Microglia–astrocyte networks

The analyses of individual glial cell types has now evolved into understanding the importance of interaction between myeloid cells and astrocytes in determining their overall contribution to tissue injury and repair. Liddelow et al. (75) showed that

activation of microglia by classical inflammatory mediators endows them with the ability to induce neurotoxic astrocytes through a mechanism mediated by the secretion of IL-1α, TNF, and C1q. Rothhammer et al. (37) showed that microglia-derived TGF-α acts via the ErbB1 receptor in astrocytes to limit their pathogenic activities and EAE development, whereas microglial VEGF-B triggers FLT-1 signaling in astrocytes and worsens EAE (32). Conversely, astrocyte-derived molecules will regulate the myeloid cells. Astrocyte-derived IL-33 promotes microglial synapse engulfment and neural circuit development (76). These findings illustrate the complexity of the astrocyte–microglial cross-talk and how this cross-talk is modulated by complex environmental factors such as the commensal flora.

In conclusion, the selected *Pillars of Immunology* paper reporting results obtained with microglia isolated from the adult human brain contributed to recognizing the dynamic properties of endogenous glial cells in the CNS and how the state of such cells regulate and mediate immune responses within the CNS. Their multitude of interactions with locally and systemically derived signals leads to a complex spectrum of phenotypes in these cells. In a therapeutic context, an ongoing challenge is how to modulate their properties to favor the desired functional response, be it in context of developmental, inflammatory, degenerative, or neoplastic disorders.

Disclosures

The authors have no financial conflicts of interest.

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