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The Role of Antigen Presenting Cells in Multiple Sclerosis

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Abstract

Multiple Sclerosis (MS) is a debilitating T cell-mediated autoimmune disease of the central nervous system (CNS). Animal models of MS, such as experimental autoimmune encephalomyelitis (EAE) and Theiler's murine encephalomyelitis virus-Induced demyelinating disease (TMEV-IDD) have given light to cellular mechanisms involved in the initiation and progression of this organ-specific autoimmune disease. Within the CNS, antigen presenting cells (APC) such as microglia and astrocytes participate as first line defenders against infections or inflammation. However, during chronic inflammation they can participate in perpetuating the self-destructive environment by secretion of inflammatory factors and/or presentation of myelin epitopes to autoreactive T cells. Dendritic cells (DC) are also participants in the presentation of antigen to T cells, even within the CNS. While the APCs alone are not solely responsible for mediating the destruction to the myelin sheath, they are critical players in perpetuating the inflammatory milieu. This review will highlight relevant studies which have provided insight to the roles played by microglia, DCs and astrocytes in the context of CNS autoimmunity.

Keywords

Multiple Sclerosis; EAE; TMEV-IDD; microglia; astrocytes; dendritic cells

1. Introduction

Multiple sclerosis (MS) is the most common idiopathic inflammatory-demyelinating disease of the central nervous system (CNS) affecting roughly 0.1% of the worldwide population, primarily young adults in North America and Europe [1]. MS is driven by myelin-specific auto-reactive T-cells that infiltrate the CNS and mediate an inflammatory response that results in demyelination and axon degradation [1-3]. Myelin is an extension of oligodendrocytes, which wraps tightly around the axon of a neuron, providing insulation for fast electrical conduction [3,4]. In the absence of myelin, neurons signal slowly or erroneously [4]. Demyelination manifests in such clinical symptoms as numbness, muscle spasms, optic neuritis, and neuropathic pain. Current therapies for MS non-specifically suppress the immune system to slow the progression of disease. New immunomodulatory therapies that induce myelin-specific T-cell tolerance are currently being developed and tested in clinical trials [5].

Antigen presenting cells (APCs) are necessary for the pathogenesis of murine models of MS [6,7]. Upon encountering myelin antigen, they mature and travel to lymph nodes where they present antigen to naïve T cells [8,9]. T cell activation and survival requires two signals

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from APCs: antigen presentation by the major histocompatibility complex [10] to the T cell receptor [11] and a secondary signal provided by the interaction of co-stimulatory molecules such as CD80 and CD86 with CD28 on T cells. During activation, T cell differentiation into mature effector $CD4^+$ T cell subsets (Th1, Th2, Th17, Treg) depends on the cytokines produced by APCs [12]. Current data suggests that MS is driven by both Th1 and Th17 subsets, although each are mechanistically different [9,13]. Once activated, T cells traffic to the brain and cross the blood brain barrier (BBB). In the brain they are re-stimulated by APCs [14], leading to disease induction and progression. As disease progresses, new myelin antigens are presented by APCs (epitope spreading), leading to subsequent activation of newly infiltrated T cells [15]. APCs are involved in multiple stages during MS pathology, thus making them important cells to study and possibly manipulate therapeutically. APC effector functions and responses are commonly studied *in vitro* and within animal models of MS.

There are currently two well-established mouse models of MS: Theiler's Murine Encephalomyelitis Virus-Induced Demyelinating Disease (TMEV-IDD) and Experimental Autoimmune Encephalomyelitis (EAE) [16,17]. TMEV is a picornavirus that is an enteric pathogen of mice and rats. Intracranial inoculation with TMEV in the IDD susceptible (SJL/J) mouse strain, results in an ineffectual immune response that limits viral titers, but does not completely clear the virus. Inability to effectively clear the virus results in chronic viral infection of the CNS leading to the induction of the myelin-specific T cell mediated autoimmune disease. Clinically, a progressive course of spastic hindlimb paralysis is evident in SJL/J mice beginning 4-5 weeks following TMEV infection. This disease course is similar to primary progressive MS [18]. Recently, a cardiovirus within the TMEV virus family has been found to infect humans, thus potentially making this mouse model even more clinically relevant [19].

EAE is the most extensively studied mouse model of MS [18], and several of these studies have lead to current treatments for MS patients [20]. It is an inducible $CD4^+$ T-cell mediated murine model of MS that exhibits relapsing-remitting phases in SJL/J mice and chronic progression in C57Bl/6 mice [21]. Disease is induced by immunization with myelin peptide and an adjuvant or by adoptive transfer of $CD4^+$ T cells isolated from immunized mice into naïve recipient animals. Like TMEV-IDD, EAE is clinically characterized by flaccid hindlimb and tail paralysis.

Although these animal models of MS are different, APCs ultimately have the same overall functions: antigen uptake, processing, and presentation along with co-stimulatory molecule expression and secretion of cytokines important for driving the proliferation and differentiation of autoreactive effector T cell subsets [22]. Understanding the contribution of CNS infiltrating dendritic cells (DC) and CNS resident microglia and astrocytes to CNS autoimmune disease is important for identifying possible therapeutic interventions. This article will review research that addresses the functions of these APC subsets known to be involved in MS, EAE, and TMEV-IDD pathology.

2. Macrophages & Microglia

Perivascular macrophages (PVMs) are an abundant cell type in the CNS, which can be distinguished from microglia based on their higher levels of CD45 and MHC class II in both rodents and humans [23,24]. Anatomically, PVMs are large, round cells that are located between the endothelial and glial basement membranes of cerebral blood vessels, thus providing a strategic location to encounter pathogens and assist in controlling innate and adaptive immune responses in the CNS [23]. Expression of MHC class II, CD80, CD86 and CD40 are highly upregulated on PVMs during EAE and MS. Activation of PVMs is also

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mediated by Th1-cytokines, IFNγ and TNFα [23]. Elimination of PVMs using clodronate containing dichloromethylene diphoshonate (Cl_2MDP) liposomes *in situ* suppresses the clinical signs of EAE, while the influx of $CD4^+$ T cells in the CNS are unaffected, suggesting that macrophages are not critical for the infiltration of T cells in the CNS [25,26]. These studies highlight a potential role for macrophages in the development of CNS inflammation and demyelination.

Microglia are the bone-marrow derived resident macrophage of the CNS. They are distinguished from peripheral macrophages by lower level expression of CD45 [27]. Under resting conditions, microglia constantly survey the CNS microenvironment, suggesting these cells are critical for maintaining CNS homeostasis [28-30]. Quiescent microglia express undetectable levels of MHC class I and II, CD80, CD86, and CD40 [31]. However, in response to inflammatory stimuli, microglia become activated and upregulate CD45, MHC and costimulatory molecule expression, phagocytic ability, and obtain the capacity to stimulate the proliferation of Th1 (IFN-γ-producing) and Th2 (IL-4-producing) $CD4^+$ T cell lines [27,32]

TLR Expression and Stimulation

Toll-like receptor (TLR) activation initiates effective induction of pro-inflammatory cytokines and chemokines, type I interferons (IFNs), and MHC and costimulatory molecules. All of which are essential for initiating and propagating host inflammatory immune responses [33]. The expression and functions of TLRs have been extensively studied in the context of recognition of CNS pathogens and autoimmunity. In response to TMEV infection, TLR 2, 3, 7, and 9 mRNA are significantly upregulated *in vitro* and *in situ* [34-36]. Microglia possess constitutive and inducible expression of surface receptors to recognize pathogens, which enable them to trigger and/or amplify an inflammatory response in the CNS [27]. It is important to note that expression patterns of TLRs in the CNS have been extensively studied from purified cells *in vitro*.

Human microglia express robust levels of TLRs1-8 but low TLR9 levels, whereas primary murine neonatal microglia constitutively express all TLRs *in vitro*. Microglia drastically upregulate several TLRs, with TLR3 being the most significantly detected, in response to direct TMEV infection and IFN-γ *in vitro* [37-40]. Expression of TLR2 and TLR3 is most abundant in MS tissue, however the cell type responsible for the increased expression has yet to be determined [37]. Collectively, these studies suggest that microglia may serve as the first line in defense in the CNS by initiating potent pro-inflammatory and antiviral responses against infectious pathogens. Using specific knockout models, future studies need to be conducted to evaluate the specific role of TLRs in microglia effector functions in the context of CNS autoimmunity. For further information about the functions of TLR signaling during CNS inflammation and autoimmunity, refer to Carpentier *et al.* 2008 [41].

Cytokine Production and Responses

Due to their ubiquitous localization within the CNS, microglia are a potent source of inflammatory cytokines and chemokines that are essential for the initiation and propagation of a CNS immune response. Microglia stimulated *in vitro* with IFN-γ, TLR stimuli, or TMEV infection, increase expression of TNFα, IL-6, and IL-1β [38,39,42]. Microglia also upregulate expression of type I IFNs in response to innate stimuli and TMEV *in vitro* [38,40,43-45]. Highly purified macrophages isolated from TMEV-infected mice at onset and chronic induced demyelinating disease serve as potent APCs similarly to microglia, however both cell types express comparable levels of inflammatory cytokines, IL-6, IL-12p40, TNFα, and inducible nitric oxide synthase (iNOS) [40]. In addition to these cytokines, microglia cultured *in vitro* increase expression and production of IL-12p40, IL-18, and IL-23p19,

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potent cytokines involved in Th1 and Th17 T cell differentiation [38,40,46-50]. In active MS lesions, increased expression of IL-23p19 is also detected in microglia/macrophages [51].

Activated microglia *in vitro* also express high levels of iNOS and produce high levels of nitric oxide (NO) in response to TLR stimuli, pro-inflammatory cytokines, or direct TMEV infection [47,52-54]. Both purified microglia and macrophages from TMEV-infected brain and spinal cord express high levels of iNOS mRNA [55], which could lead to oligodendrocyte and myelin damage propagating demyelination. In acute MS lesions, iNOS is localized in macrophages, but absent in chronic MS lesions [56]. Collectively, the production of cytokines by both microglia and macrophages are critical for directing the inflammatory environment within the CNS.

Although the functions of IFN-γ, TNF-α, and IL-12 have been extensively studied in MS and its animal models, research efforts are now focused on understanding how IL-17 and Th17 cells contribute to the development and progression of MS and EAE. Th17 cells are characterized by the production of IL-17A, IL-17F, and IL-22 [57]. The differentiation of murine $CD4^+$ T cells into Th17 cells is dependent on signals from IL-6, tumor growth factor-beta (TGF-β) and IL-1; while human $CD4^+$ Th17 cells requires TGF-β in combination with IL-1β and IL-6 *or* IL-23 [58,59]. IL-23 is required for the survival and maintenance of these differentiated cells [57]. Microglia display constitutive and inducible mRNA expression of IL-23p19, IL-12/23p40, and IL-12p35, and their receptors, IL-23R and IL-12Rβ1 in response to LPS or IFNγ *in vitro* [47]. During EAE, expression of IL-23p19 in CNS $CD11b^+$ cells (microglia, macrophages, and dendritic cells) peaks early in disease, suggesting that expression of IL-23p19 by these cells is critical for the initiation of CNS inflammation prior to peripheral T cell infiltration [47].

Studies performed by Das Sarma et al., examined IL-17 and IL-17R expression in the CNS during EAE [60]. They demonstrated that IL-17R is constitutively expressed *in vivo* in the CNS at very low levels, and is highly upregulated in the spinal cord during EAE [60]. The cellular source of IL-17R has yet to be determined *in situ*, however glial cells may serve as a potential source for its expression during CNS inflammation. Purified microglia *in vitro* constitutively express IL-17RA mRNA, however express very low levels of IL-17R protein [60]. Despite low protein expression of IL-17R, microglia are capable of efficiently responding to IL-17 stimulation, and how this relates to the function of microglia in the induction and progression of disease has yet to be determined.

T Cell Recruitment

Chemokine expression by microglia and macrophages is critical for the recruitment of leukocytes to the CNS. Microglia cultured *in vitro* increase expression of CCL2-5 and CXCL3 following stimulation with TMEV [38,39,42]. Treatment with IL-17A stimulates significant production of CCL2, CCL12, CXCL1, and CXCL2 from both microglia and astrocytes, suggesting a potential signaling cascade to regulate chemokine expression during inflammatory, demyelinating disorders [60]. The importance of glial produced chemokines in EAE is highlighted in a study by Dogan *et al.* Bone marrow chimeric and $CCL2^{-/-}$ mice were used to further evaluate the significance of glial-derived production of CCL2. CNS-$CCL2^{-/-}$ mice develop significantly reduced EAE disease onset, progression, and demyelination, and had decreased levels of iNOS- and TNFα-producing myeloid cDCs ($CD11b^+CD11c^+$) and macrophages ($CD11b^+CD11c^-$) in the spinal cords of mice during EAE. This demonstrates CNS glial-produced CCL2 is required for the recruitment of iNOS and TNFα-producing DCs and macrophages to obtain optimal EAE disease [61]. In MS plaques, microglia/macrophages *in vivo* are also significant producers of CCL2, CCL3,

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CCL4, and CCL5 [62,63]. Thus, microglia and macrophages are important regulators of recruiting infiltrating leukocytes into the CNS.

Antigen Processing

Following activation, microglia are responsible for the removal of cellular debris and pathogens during CNS injury, inflammation, and infections, which can be correlated to antigen processing and presentation to T cells [64]. Rodent and human microglia *in vitro* phagocytize whole proteins and peptides of myelin [65,66]. Several *in vitro* studies have shown that myelin phagocytosis by microglia and macrophages triggers release of proinflammatory cytokines and nitric oxide [66-68], suggesting that phagocytosis of myelin could enhance neuroinflammation. Conversely, clearance of myelin debris is necessary for remyelination, suggesting a dual role for microglial phagocytosis [69].

T Cell Priming

To initiate T cell activation, APCs must deliver two-signals: one from antigenic peptides bound to MHC, and a second from a co-stimulation signal. In the normal CNS, resident microglial cells express negative or low levels of MHC molecules [64]. During TMEV-IDD and EAE, microglia highly upregulate MHC I and II antigens *in vitro* and *ex vivo* [38,40,44,55,70]. IL-17 in combination with IFN-γ upregulates cell-surface expression of MHC class II on microglia, however IL-17 alone has minimal effects, suggesting that there may be synergistic effects between IL-17 and IFN-γ for initiating $CD4^+$ T cell responses and propagating inflammation within the CNS [71]. In MS brain tissue, reactive microglia localized in active lesions had enhanced expression of MHC class II molecules, HLA-DR, -DP, and –DQ [72]. Microglia constitutively express low levels of CD80 and CD86 mRNA, but expression is upregulated in response to inflammatory stimuli and TMEV virus infection *in vitro* and *ex vivo* [38,40,53,65,66,73]. CD40 expression is upregulated on microglia following stimulation with IFN-γ or TMEV *in vitro* and *ex vivo* [38,40,53,65,66,73]. $CD40^{-/-}$ microglia fail to become fully activated at peak EAE disease, which correlates with a reduction in encephalitogenic T cells [74].

Microglia also present antigen to and activate $CD4^+$ Th1 and $CD8^+$ T cells in response to direct inflammatory stimulation, indicating microglia play a role in the activation of and/or cytotoxic cell lysis by peripheral T cells infiltrating the CNS [31,38,40,75-77]. IFN-γ-activated and TMEV-infected murine microglia are also capable of presenting exogenous and endogenous myelin and viral peptides to $CD4^+$ T cells *in vitro* and *ex vivo* [31,38,44,77,78].

Potentially due to their lower expression of MHC class II, microglia serve as poor activators of naïve T cells. For example, purified microglia and macrophages isolated from mice with EAE are able to induce the proliferation and IL-2 production of naïve $CD4^+$ T cells however only at a relatively high APC:T cell ratio [6]. Rather, these cells may play a more important role in the reactivation of T cells infiltrating into the CNS during MS. These studies demonstrate that microglia increase expression of co-stimulatory and MHC molecules and are capable of presenting antigen to T cells more effectively compared to astrocytes, however less efficiently than DCs [22,31].

Regulation

A number of regulatory mechanisms exist between T cells and APCs, which elicit T cell dysfunction during autoimmunity and viral infections. Programmed death receptor-1 and its ligands, programmed death-ligand 1 (B7-H1/PD-L1) and programmed death-ligand 2 (B7-DC/PD-L2) are integral players in inhibiting ongoing T cell responses [79]. Blocking the signaling between B7-H1:PD-1 helps restore “exhausted” T responses, resulting in increased

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T cell function during viral infections [80]. In the context of autoimmunity, inhibition of B7-H1:PD-1 signaling has a detrimental effect due to the lack of control of autoreactive T cell responses. B7-H1 expression in microglia is constitutive and inducible by IFN-γ stimulation *in vitro* [81,82]. Additionally, CNS-infiltrating monocyte/macrophage/dendritic cells ($CD11b^{+}CD45^{hi}$) and CNS-resident microglia display enhanced expression of B7-H1 during remission of EAE compared to acute phase of EAE, however both cell types lack both constitutive and inducible expression of B7-DC [82]. Blockade of B7-H1 on CNS-infiltrating macrophages and microglia isolated from mice with $PLP_{139\text{-}151}$-induced EAE reduced $CD4^{+}$ T cell proliferation and production of IFNγ and IL-17 *ex vivo* [81]. B7-H1 expression was found in chronic active MS lesions [83]. Collectively, these studies identify a novel interaction for the negative regulation of myelin-specific T cells by both microglia and macrophages.

Requirement of Microglia in EAE

We have provided several lines of evidence highlighting the function of microglia in the initiation and regulation of the immune response within the CNS. However, there are few studies that demonstrate the requirement for microglia in EAE pathology. To determine the function of microglia *in vivo*, Heppner *et al.* developed transgenic mice to specifically ablate microglia using a system in which Herpes simplex thymidine kinase expression was driven under the CD11b promoter (CD11b-HSVTK) [84]. To ensure specificity bone marrow chimeras were made such that only microglia expressed CD11b-HSVTK. Depletion of microglia following treatment with gancyclovir (GCV) during EAE resulted in reduced disease onset, progression, and demyelination. Production of TNF-α, NO, and CCL4 was inhibited in brain slice cultures in the presence of GCV in CD11b-HSVTK transgenic mice, suggesting that microglial-derived inflammatory production of cytokines, chemokines, and NO, promotes the recruitment and activation of leukocytes into the inflamed CNS [84]. While results from this study demonstrated the importance of microglia in contributing to disease severity, a direct link for propagating T cell proliferation *in vivo* has yet to be demonstrated due to the lack of technological advances.

To further understand microglia during EAE, Adams *et al.* identified a novel mechanism for their activation [85]. During on-going $PLP_{139\text{-}151}$-induced EAE, antibody mediated depletion of fibrin, a protein deposited in the perivascular cuffs following BBB disruption, resulted in fewer relapses compared to untreated mice. Additionally, immunization with a specific fibrinogen peptide, fibrin $\gamma^{377\text{-}395}$, prior to disease induction resulted in a reduction in $iNOS^{+}$ microglia, but had no effect on T cell infiltration [85]. Upon fibrin stimulation *in vitro*, anti-CD11b neutralizing antibody blocked microglial activation. Therefore, blockade of fibrinogen-CD11b interactions may be a potential therapeutic target to inhibit microglial activation and suppress EAE disease.

In conclusion, both microglia and macrophages display a wide range of effector functions essential for controlling T cell responses which contributes to the onset and progression of MS and its murine models.

3. Dendritic Cells

Dendritic Cells are a class of bone marrow-derived cells, arising from both myeloid and lymphoid progenitors. They are essential in the coordination of both the innate and adaptive immune responses. Classified as professional APCs, under steady-state conditions their distribution throughout tissue is diverse; they are present in lymphoid and non-lymphoid tissue as well as in circulation [86]. Upon activation through TLR signaling or encounter with antigen (Ag), DCs can migrate from resident tissues or sites of inflammation to the lymph nodes (LN) through their differential expression of chemokine receptors including

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CCR7 [87,88]. Activation of DCs leads to their maturation, where they express increased levels of MHC class II as well as co-stimulatory markers (CD80, CD86, CD40). These modifications allow them to become efficient at presenting cognate Ag to naïve as well as memory T cells, which is critical for launching the adaptive immune response.

DCs are a diverse cell type made up of several subsets. These are classified by their expression of a variety of cell surface markers, as well as possess various functional capacities. Two main subsets of DCs are present in the steady state: conventional DCs (cDCs) and plasmacytoid DCs (pDCs). Conventional DCs are identified by their expression of the integrin CD11c and can express both CD11b and CD45. They can be further subdivided into $CD8^+$ and $CD8^-$ subsets. Within the spleen cDCs are located in the marginal zones and T cell areas, and are very efficient at priming and promoting differential T cells responses. pDCs are $CD11c^{int}$ and express the B cell marker B220. PDCs produce large amounts of Type I interferons after viral infection and are circulatory in nature, migrating to lymph nodes after activation. However, they are less efficient at priming T cell responses than cDCs. For an extensive review on DC subsets and their development, please see review by Wu and Liu [86].

Ontogeny

The accumulation of DCs within the CNS [89-95] is noteworthy, given the immunoprivileged nature of the CNS. Since the CNS has resident APCs (microglia and astrocytes), infiltrating DCs may seem excessive. Initially it was important to determine the origin of the DCs—whether they derived from CNS resident precursors or whether they were indeed infiltrating from the periphery. It has been determined that within the context of CNS inflammation, $CD11c^+$ DCs are preferentially derived from the bone marrow [9,96]. This was demonstrated in studies using bone marrow chimeras of congenic mice. In these experiments, $CD11c^+$ cells were shown to be derived from donor cells (hematopoietic lineage) rather than the recipient (radioresistant) CNS cells [7,9]. Thus, evidence indicates that CNS DCs accumulating during inflammation are *infiltrating* rather than proliferating from CNS precursors.

Recruitment

The presence of DCs within the CNS is apparent, yet recruitment of DCs to the CNS has not been well explored. *In vitro* studies using primary cultures of brain microvascular endothelial cells (BMEC) showed increased levels of DC transmigration with the addition of the chemokine MIP-1α (CCL3). Migration by DCs across the BMECs was facilitated by DC secreted matrix metalloproteinase (MMP) 9 [97]. It was also demonstrated that migration of DCs also led to their maturation, resulting in increased expression of costimulatory molecules. In these experiments, MIP-1α appears to be involved in *in vitro* migration; understanding the role of this chemokine should be verified *in vivo*. Although migration of DCs within peripheral tissues has been well studied, mechanisms of DC recruitment into the CNS and across the BBB continues to be an area of ongoing research.

Localization studies of DCs in both mouse models and human MS patients have provided clues to the function of DCs within the CNS. In the inflamed tissue, DCs are found within inflammatory foci [91,94,98,99]. Interestingly, different subsets of DCs may preferentially home to different regions of the brain. “Myeloid” cDCs ($CD11c^+CD11b^+CD8$-) were found to cluster with $CD4^+$ T cells in the perivascular inflammatory foci [9,96]. Comparatively, macrophages were more marginalized [9]. This organization is reminiscent of secondary lymphoid tissue structure. In addition, mDCs and macrophages were found throughout the cerebellum and spinal cord, while pDCs accumulated more superficially in the cerebellar meningeal area [9].

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Not only are DCs seen in mouse models of MS, but the presence of DCs in MS lesions has also been demonstrated [100,101]. Serafini *et al.* found that both immature and mature (DC-SIGN$^+$) DCs were present in the meninges and parenchymal lesions of patients with both primary and secondary progressive MS [101]. In non-inflamed tissue, DC-SIGN$^+$ DCs were found lining the blood vessels, suggesting they could be one of the first cells to encounter migrating T cells [7]. MDCs from the cerebral spinal fluid of patients with MS have been found to be more mature, expressing higher levels of accessory molecules (HLA-DR, CD80, CD86 and CD40) [102] and also producing higher levels of the pro-inflammatory cytokine IL-6 [103]. DCs that had engulfed MBP were also found in close proximity to proliferating CD8$^+$ T cells. In addition, many of these DCs expressed CCR7, a chemokine receptor often associated with maturation. Thus, it was concluded that immature DCs are able to migrate to the CNS where they are able to mature as well as present autoantigen to infiltrating T cells, thereby propagating the autoreactive inflammation [101].

Antigen Processing and Presentation

Functionally, DCs are essential for priming both CD4$^+$ and CD8$^+$ T cell responses. Upon their activation through TLR recognition, they are capable of mediating Ag presentation as well as secreting cytokines that can influence the developing adaptive response. For instance, in viral settings, pDCs produce large amounts of type I IFNs, while CD8$^+$ cDCs have been shown to secrete IL-12 and promote a Th1 environment [104]. Classically, T cell activation occurs in the LN where DCs migrate after Ag uptake in peripheral tissues.

Though “mature” DCs are detectable in the inflamed brain, conflicting results from early studies suggested CNS infiltrating DCs were very poor T cell stimulators, or even suppressed T cell function [91,94,98]. We and other groups have addressed these experimental questions and the data indicates a strong role for DCs in CNS inflammation. Indeed, CD11c$^+$ DCs are the only APC required for the initiation of adoptive transfer EAE. Greter *et al.* used H2-Ab1 (MHC class II) knockout mice, where H2-Ab1 expression is driven by the CD11c promoter (CD11c-H2-Abi/H2-Abi$^{-/-}$). In this system MHC class II is exclusively restricted to CD11c$^+$ DCs. Following adoptive transfer, mice were susceptible to EAE [7], demonstrating that CD11c$^+$ DCs alone are sufficient for the development of disease.

Furthermore the number of DCs is correlated with disease severity. In a separate set of studies, mice were treated with Flt3L which binds to a receptor on DCs inducing their expansion [105]. Mice treated with Flt3L had increased numbers of DCs and greater disease score. Inhibiting this signal with specific inhibitors reduced clinical signs of disease [106]. The evidence that CD11c$^+$ DCs are the only cell type required for disease induction, and their increased numbers correlates with increased disease, highlights the importance of these cells in promoting CNS autoimmune pathology.

Within the CNS, it is likely that DCs are able to process and present local Ag to infiltrating T cells. Evidence for this comes from our previous studies which demonstrated that CNS infiltrating CD11c$^+$ F4/80$^-$ DCs (“non myeloid”), along with macrophages, stimulated naïve auto-reactive T cells with or without the addition of exogenous myelin peptide in both EAE as well as TMEV-IDD [6]. Indeed, the CNS infiltrating DCs were the most efficient at stimulating naïve T cells. This demonstrates that DCs migrating into the CNS are able to acquire, process and present endogenous myelin proteins. Although this study demonstrated the role of DCs in the propagation of the CNS inflammation, it did not address their participation in initiation.

Clues to the initiation of myelin-specific T cell activation by DCs comes from several studies addressing the presence of myelin and neuroantigen containing APCs in the cervical

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LNs (cLNs) in human MS patients and both mouse and monkey models of EAE [107-110]. These studies have demonstrated the presence of myelin antigen contained within APCs in cLN of MS patients and monkeys affected by EAE [107,108]. The presence of myelin within the cLN was found to be specifically due to demyelination. As well, APCs within the cLNs have been found to contain neuroantigens, although, these were seen in patients with MS as well as healthy controls [110]. Although the CNS is considered an immunopriveleged site, cLN are considered to be the "draining" lymph nodes for the CNS. Interestingly, a recent study showed that myelin debris and APCs containing myelin are also present in the meninges and perivascular spaces of MS patients. These DCs could potentially be *en route* to the cLN where they could present Ag to T cells [109]. These studies provide insight into the potential mechanism of the peripheral priming of auto-reactive T cells. Future studies should address the how DCs migrate from the CNS into the cLN.

As previously discussed in this review, the role of Th17 cells during MS has become newly appreciated. A study from our lab was the first to demonstrate that CNS "myeloid" ($CD11c^+CD11b^+$) DCs were able to not only activate naïve PLP139 specific $CD4^+$ T cells, but also preferentially skewed them to differentiate into Th17 effectors [9]. Although mDCs resembled $CD8^+$ DCs and pDCs with respect to activation markers (MHC class II, CD80, CD86), they were far more efficient at priming naïve T cell responses. In concordance with CNS DCs "driving" a Th17 response upon stimulation with CD40, CNS DCs have also been shown to produce the appropriate Th17 "driving" cytokines: TGF-β, IL-6 and IL-23 [9,96]. Future studies should identify signals that induce CNS infiltrating DC mediated T cell skewing so that this may be exploited therapeutically.

It is clear that DCs indeed play a critical role in mouse models of CNS inflammatory demyelinating disease as well as in MS. DCs are the most efficient APC population at priming naïve T cells. This property, although normally beneficial, becomes quite pathogenic in the context of CNS autoimmune inflammation.

4. Astrocytes

Astrocytes are non-traditional brain-resident APCs. They are the most abundant cell type of the brain and serve a variety of functions, primarily unrelated to APC effector functions. Briefly, they are involved in maintaining the BBB, metabolizing glutamate, stabilizing the extracellular concentration of potassium, and producing trophic survival factors for neurons and other glia [111]. Astrocytes have a star-like morphology and reside relatively stationary in both the gray and white matter of the CNS [111]. Astrocytes are not commonly associated with immunological responses, but due to their abundance in the CNS, their response to inflammation must be considered. It is well accepted that their role in CNS immunity is largely regulatory [112] while their contribution and capacity to present antigen and activate T-cells are still controversial.

TLR Expression

Human and murine astrocytes only express one TLR constitutively, TLR3, which detects double-stranded RNA (ds-RNA) common to viruses [113-116]. Both intracellular and extracellular expression of TLR3 has been detected in human astrocytes and upregulation can be triggered by IFN-γ or IL1-β [113]. PolyI:C stimulation, which synthetically mimics ds-RNA, induces upregulation of TLR2-4 *in vitro* as well as several growth and neuroprotective factors [115,117,118]. Astrocytes also express a number of other pattern recognition receptors [119]. TMEV infection induces an array of cytokine and chemokine secretion via TLR3 activation [120]. Interestingly, TLR3 activation plays a major role in the astrocytic response to polyI:C but not TMEV infection; conversely ds-RNA-dependent protein kinase PKR is more important for responding to TMEV, but not polyI:C stimulation

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[121]. These data imply different mechanisms in astrocytic responses to viral stimuli, which may be important for understanding the induction and progression in TMEV-IDD and MS.

Cytokine Production

Whether or not astrocytes are successful at presenting antigen to T cells during MS, they can provide an environment that promotes T cell activation. Astrocytes are capable of producing IL-12 (Th1), IL-4 (Th2), IL-23 (Th17), IL-6 and TGF-β (Th17), each of which can drive naïve T cells into a different T cell subtype in the presence of antigen presentation [122]. However, it is currently unknown what impact astrocytic-produced cytokines have on T cell differentiation during MS.

T Cell Recruitment

Although astrocytes are weak APCs, they serve an important role in regulation and T cell recruitment by producing potent levels of cytokines and chemokines. Astrocyte processes surround blood vessel endothelial cells, making astrocytes major players in recruiting leukocytes from the periphery. Inflammation induces astrocytes to produce cytokines that increase the blood brain barrier permeability, allowing easier infiltration by leukocytes [119]. In response to IFN-γ, TNF-α, and TMEV infection astrocytes upregulate ICAM-1 and VCAM-1 adhesion molecules which aids in leukocyte recruitment [123-125]. In response to IL-17, astrocytes produce CCL2, CCL12, CXCL1, and CXCL2, a distinctly different profile compared to IFN-γ stimulation [13,60]. Human and murine astrocytes also recruit monocytes and T cells by producing significant amounts of CCL2, CCL5, and CXCL10 in response to TMEV infection and polyI:C stimulation [113,115,126]. Astrocytes produce CCL2 during multiple phases of EAE as well [127]. Interestingly, TNF-α and IL-1β stimulate astrocytes to secrete chemokines that attract immature, but not mature, DCs. [128] Thus, astrocytes could potentially be recruiting more effective APCs into an environment full of myelin debris, at the same time myelin-specific T cells requiring re-stimulation are recruited, further propagating disease. Kang *et al* recently determined that EAE disease progression was inhibited and leukocytes were not recruited in a nestin-specific (astrocytes, neurons, oligodendrocytes) knockout of act1, an important protein in the IL-17R signaling complex [13]. They suggested that astrocytes were most likely the cellular compartment responsible for recruiting leukocytes based on their act1-dependent chemokine production in response to IL-17 *in vitro*. These data imply that astrocytes are necessary for disease progression in Th17-mediated disease due to their chemokine response to IL-17. Further investigation is necessary to determine whether responses to IL-17 in oligodendrocytes and neurons contribute to leukocyte recruitment.

MHC and Co-stimulatory Molecule Expression

A number of studies have focused on characterizing the ability of astrocytes to express MHC class I and II, and co-stimulatory molecules under normal and inflammatory conditions to determine if they are capable of presenting antigen to T cells [70,129-133]. MHC class II is expressed in two known environments in humans: MS lesions [134] and IFN-γ-stimulated astrocytes *in vitro* [135]. Murine astrocytes also express very low levels of MHC class II but upregulate expression upon IFN-γ stimulation [70,129,130,136]. TNF-α inhibits IFN-γ-induced MHC class II upregulation without affecting the increased expression of class II major histocompatibility complex transactivator (CIITA), a transactivating protein necessary for MHC class II transcription [124]. For a review on CIITA regulation of MHC class II expression in astrocytes, see Dong and Beneviste 2001 [137]. IL-1α, TGF-β, and serotonin receptor agonists also inhibit IFN-γ-induced MHC class II upregulation in astrocytes [138-140]. As for MHC class I expression, astrocytes normally express relatively low levels, but upregulate it in response to viral infection, yet not as fast or robustly as microglia [131].

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Co-stimulatory molecule expression is less straightforward. Two groups have demonstrated that murine astrocytes constitutively express CD86 at low levels, but upregulate CD80 and CD86 in response to IFN-γ stimulation [129,141]. Soos *et al* did not observe CD80 upregulation upon IFN-γ stimulation in cultured astrocytes, but noted a correlation between T cell activation and astrocytic expression of CD80/86 molecules [133]. Similarly, Aloisi *et al* did not detect IFN-γ-induced upregulation of CD80 or CD86 expression *in vitro*, but determined that IFN-γ-stimulated astrocytes were still capable of inducing differentiation of T cells into a Th2 phenotype [31]. However, IFN-γ-stimulated murine astrocytes *in vitro* may not behave the same way during EAE or MS. Indeed, astrocytes do not express CD80, CD86, or CD40 during EAE [132,142]. Furthermore, researchers studying human astrocytes *in vitro* reported no constitutive or IFN-γ-induced expression of CD80/86 molecules [53]. However, a more recent study established that astrocytes in chronic MS lesions do express CD80 and CD86 [143]. Human fetal astrocytes upregulated CD40 expression in response to TNF-α, IL-1β, IFN-γ and LPS *in vitro* [144]. Although expression of co-stimulatory factors is necessary for successful T cell activation, the best analysis of APC function is their interaction with T cells.

T Cell Priming

In TMEV-IDD, astrocytes have been reported to be the major CNS-resident cell type responsible for viral persistence [145], indicating that astrocytes must not successfully present antigen to $CD8^+$ T cells for efficient viral clearance. However, there is convincing evidence that virally infected astrocytes form both Kupfer and non-Kupfer immunological synapses with $CD8^+$ T cells *in vivo* and *in vitro*, induces a polarizing morphological change uncommon to uninfected astrocytes [146-148]. Interestingly, there are differences in astrocytic antigen presentation between murine strains. Unstimulated BALB/c, but not C57Bl/6 astrocytes, were capable of stimulating $CD8^+$ T cells *in vitro* in a peptide dose-dependent way [129]. Yet TMEV-resistant (C57Bl/6) astrocytes were more efficient in processing and presenting viral antigen than TMEV-susceptible (SJL/J) astrocytes after TMEV infection and IFN-γ stimulation *in vitro* [126]. Infected or stimulated TMEV-resistant astrocytes had a higher upregulation of MHC class I, vascular cell adhesion molecule 1 (VCAM-1), and intercellular cell adhesion molecule 1 (ICAM-1) compared to TMEV-susceptible astrocytes [126]. These differences between C57Bl/6 and SJL/J astrocytes could potentially regulate susceptibility and resistance to the development of demyelinating disease.

In EAE, naïve T cells are first introduced to myelin antigens in the periphery, and are required to be reactivated upon entering the CNS [14]. It is possible that astrocytes may be in part responsible for this reactivation of myelin specific T cells. Several studies have demonstrated that IFN-γ-stimulated astrocytes are capable of inducing Th1 differentiation and proliferation of naïve myelin-specific T cells [114,130,133,149-151]. Interestingly, only $PLP_{139-151}$ specific T cells activated by IFN-γ-stimulated astrocytes induced EAE by adoptive transfer and not those T cells specific to subdominant PLP epitopes [130]. This data suggests that astrocytes may not be involved in epitope spreading, but may play a role in the initial inflammatory response. MBP-specific T cell proliferation induced by IFN-γ-stimulated astrocytes was blocked by antibodies against IL-12/23 p40, indicating that astrocytes are capable of maintaining activated Th1 and Th17 cells [149].

Astrocytes may not be as efficient at priming T cells *in vivo* as these data purport. For instance, IFN-γ-stimulated astrocytes expressing human MHC (HLA-DR2 & 4) were efficient in presenting MOG peptide but not native protein, [150] signifying a deficiency in antigen processing or peptide loading. Given the right pro-inflammatory environment with available peptide, astrocytes are capable of priming myelin specific $CD4^+$ and $CD8^+$ T cells,

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but whether or not this specific environment occurs during MS or EAE requires more investigation.

Regulation

Similar to professional APCs, astrocytes react to injury and infections in a stimulus-specific way. Astrocytes are capable of modifying the response of all other cell types in the CNS primarily through the production of cytokines, making them powerful regulators of both immune responses and repair. Astrocytes can both enhance and down regulate the activation of microglia. For instance astrocytic production of GM-CSF and macrophage colony stimulating factor (M-CSF) is required for microglial antigen presentation, whereas TGF-β production can down regulate microglial activation and MHC class II expression [152-154]. During EAE and MS, astrocytes produce immunosuppressive TGF-β and IL-10 [154]. Astrocytes also contain inflammation by forming a physical barrier called a glial scar, characterized by morphological and gene expression changes induced by inflammation [155]. Neurons are vulnerable during immune responses, however astrocytes commonly upregulate neuroprotective genes such as neurotrophin-4, ciliary neurotrophin factor, and nerve growth factor after presenting antigen to MBP-specific Th1 and Th2 cells and in response to viral infections [118,151].

Peripheral immune responses are largely regulated by death receptor-mediated cell death. Astrocytes constitutively express high levels of Fas and FasL, however they are unusually resistant to death receptor induced apoptosis, allowing them to regulate Fas-expressing cells such as activated T cells while surviving most Fas-mediated signals [156,157]. Fas signaling is cytotoxic to microglia, whereas astrocytes survive and respond by producing inflammatory chemokines [158]. Thus, astrocytes may be more important for regulating infiltrating immune responses by attenuating inflammation.

Astrocytes are dynamic cells that are necessary for multiple cellular processes in the CNS. They may be inefficient at presenting antigen, but they are resilient responders to inflammation, providing both proinflammatory and protective regulatory responses.

5. Conclusions

The protection of the CNS via CNS-resident cells and infiltrating immune cells is paramount for the survival and defense of an individual. Regulation of these cells and their responses is important for maintaining a homeostatic environment. However, under aberrant conditions CNS inflammation can persist and lead to the chronic development of autoimmunity. APCs within the CNS are critical for host defense against pathogens, however they can contribute to a heightened inflammatory milieu and thus can also be harmful to oligodendrocytes and myelin. Microglia have been demonstrated to upregulate classical APC machinery (*i.e.* MHC class I and class II), as well as secrete inflammatory cytokines such as TNF-α. Though they are not the most numerous cell type within the CNS, their ability to influence the immune response indicates their potency. Dendritic cells, though not CNS resident cells, are emerging as a key player in the priming autoreactive T cells both within the periphery as well as within the CNS. Additionally, they appear to be able to direct an inflammatory Th17 type response, which is newly associated with chronic autoimmune diseases such as MS. Astrocytes are the most numerous cell type in the CNS and are crucial for maintaining BBB integrity, however can be harmful via their ability to secrete inflammatory chemokines directing the recruitment of infiltrating immune cells that can mediate damage to oligodendrocytes and their myelin. Future studies targeted at prevention of APC effector function may lead to the development of effective therapies for limiting chronic CNS inflammation and autoimmunity.

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